

1998

# Supplemental Organic Chromium for Dairy Calves.

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**SUPPLEMENTAL ORGANIC CHROMIUM  
FOR DAIRY CALVES**

**A Dissertation**

**Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy**

**in**

**The Department of Dairy Science**

**by**

**Christopher L. DePew  
B.S., Purdue University, 1990  
M.S., Louisiana State University, 1993  
May 1998**

**UMI Number: 9836866**

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## ACKNOWLEDGMENTS

I would like to express my sincere thanks to Dr. L. Dwain Bunting, my advisor and friend for his invaluable support, guidance, and patience during my doctoral program. My appreciation also goes to the members of my advisory committee, Drs. J. Marcos Fernandez, Michael J. Keenan, R. W. Adkinson, and R. K. Cooper, and Drs. Donald L. Thompson, Jr., and L. Lee Southern. It is from these individuals that I have learned what it is to be a scientist and for that I owe them my deepest gratitude.

A special thanks is extended to Dr. Kaz Ingawa for assistance with statistics, but mostly for his friendship. To Laura Gentry, Angelica Chapa, Therisa Knowles, and Lorie Smith, I am eternally indebted to you for your continuous encouragement and assistance. To Dr. T. W. White and Fernando Vazquez for providing advice and suggestions in ways that keep life interesting and fun. In addition, I would like to thank the many graduate and undergraduate students who also assisted in blood collection, animal handling, and laboratory analysis: Terry Ward, Danny Gantt, Robert Fornea, Les Walz, Todd Teriffa, and Angie Louque.

To the two who have guided and shaped my identity and without whom I would not have achieved the goals I set so many years ago, I would like to extend my love and admiration to my parents Larry and Peg DePew as well as my siblings Tony, Andy, and Katie.

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## LIST OF ABBREVIATIONS

ADG	average daily gain
AUC	area under the response curve
BW	body weight
CP	crude protein
Cr	chromium
CrCl <sub>3</sub>	chromium chloride hexahydrate
CrNIC	chromium nicotinate
CrP	chromium picolinate
DM	dry matter
DMI	dry matter intake
GLM	general linear model
GTF	glucose tolerance factor
h	hour
IGF-1	insulin-like growth factor-1
IVGTT	intravenous glucose tolerance test
IVICT	intravenous insulin challenge test
i.v.	intravenous
min	minute
NEFA	nonesterified fatty acids
PLT	propionate loading test
ppm	parts per million

**RIA**

**radioimmunoassay**

**s**

**second**

**TG**

**triacylglycerol**

**wk**

**week**

## ABSTRACT

In Exp. 1, 42 calves were fed milk replacer and then starter either with or without 1 ppm supplemental chromium (Cr) as Cr-picolinate. Neither Cr nor sex affected growth performance. With the exception of plasma nonesterified fatty acid concentrations (NEFA), weekly plasma metabolites were not affected by Cr or sex. Pre- and postfeeding plasma NEFA concentrations were lower in Cr-fed calves, but were not affected by sex. All calves seemed to become less insulin sensitive with age, as plasma glucose became lower and insulin higher as calves became older. During i.v. glucose tolerance tests (IVGTT), calves cleared glucose faster at 2 compared with 8 weeks of age but Cr did not affect clearance at either week. At 2 weeks of age, heifers cleared glucose faster than did bulls. Plasma glucose increases after an i.v. propionate load were greater in heifers than in bulls but were not affected by Cr.

In Exp. 2, 34 calves were fed milk replacers either without (BF) or with (HF) added fat and either with or without 1 ppm of dietary Cr as Cr-nicotinate. Neither Cr nor fat affected performance; except, weight gain was greater in HF-fed calves during the period calves received milk alone. Weekly plasma glucose, insulin, and triacylglycerol concentrations were not affected by Cr; however, plasma insulin concentrations tended to be lower in Cr-fed compared with control-fed calves. Weekly NEFA declined in a similar manner for Cr-fed and control-fed calves; however, overall, NEFA concentrations were greater in Cr-fed calves. Weekly cholesterol concentrations were greater in Cr, HF-fed calves and lower in Cr, BF-fed calves compared with controls. Added dietary Cr or fat had minimal effects on plasma metabolites and hormones measured after milk replacer feeding.

Using data generated by the IVGTT, a computer modeling procedure predicted that insulin sensitivity was increased in Cr-fed calves but reduced in HF-fed calves compared with controls. Overall, data suggested that Cr had little effect on metabolism or growth performance, but may have improved insulin sensitivity, with the most notable effects occurring in the initial weeks of life.



## CHAPTER I

### INTRODUCTION

The essentiality of dietary Cr for man and many laboratory species has been reasonably well established (Anderson, 1987; Mertz, 1993). The most convincing evidence comes from long-term total parenteral nutrition studies in humans (Jeejeebhoy et al., 1977; Freund et al., 1979; Brown et al., 1986) and from feeding low-Cr diets to laboratory animals (Schroeder et al., 1963a,b; Mertz et al., 1965a,b; Hopkins, 1965; Onkelinx, 1977; Kraszeski et al., 1979; Jain et al., 1981). These studies demonstrated that a Cr deficiency impairs glucose tolerance and that supplementation of physiological amounts of Cr reestablished normal glucose tolerance.

The only established metabolic role of Cr is its function in the glucose tolerance factor (GTF), where Cr is believed to play a physiological role as a co-factor for facilitating insulin's attachment to its receptors (Mertz, 1979; Offenbacher and Pi-Sunyer, 1988; Mertz, 1993). In monogastric species, insulin has a multiplicity of effects within the body: it acts on a variety of tissues including muscle, adipose, liver, and mammary, and alters a variety of processes including membrane translocases, enzyme activity, and enzyme amounts (Vernon and Sasaki, 1991). More specifically, insulin enhances glucose uptake by adipose and muscle cells, enhances amino acid uptake by muscle cells, stimulates protein synthesis, and inhibits protein degradation; thus, in general, insulin promotes tissue growth (Granner, 1996). In ruminants, insulin has many similar effects, but these effects, especially on adipose and muscle tissue, are generally less pronounced than those observed in monogastric animals. The smaller response to insulin of ruminants tissues have been attributed to the

pattern of feeding and the action of the rumen, both of which minimize the surge of nutrients, especially glucose, into the blood stream. Because the influx of nutrients into the blood following a meal is less episodic in ruminants, there appears to be less need for tissues to be as acutely sensitive to insulin. In other words, the need for insulin to act rapidly to move nutrients into storage (i.e., glucose and amino acids into muscle and glucose and lipids into fat) is not as much of a priority. Nevertheless, insulin in ruminants increases on feeding (Bassett, 1975), and development of diabetic-like conditions can be as severe in ruminants as in monogastrics (Jarrett et al., 1974). Because Cr is a component of the GTF-complex, because the GTF is important for optimum insulin effectiveness, and because insulin is involved in regulating a multiplicity of metabolic events, it seems plausible that any system upon which insulin has an influence would work most efficiently when an animal's Cr requirement is satisfied.

As alluded to above, ruminants derive small amounts of glucose from intestinal absorption and the role of insulin in these animals is perhaps less well defined than in the nonruminant animal (Brockman, 1986). By contrast, the pre-weaned calf continues to absorb glucose primarily through the small intestine until its rumen begins to function as a result of consuming dry feed (Quigley et al., 1991). Therefore, until such time, the calf's absorption of nutrients is more like that of a monogastric than that of an adult ruminant. Evidence has shown that tissue sensitivity to insulin is greater in the preruminant compared with the adult ruminant animal, but less than that of the monogastric (Thiebaud et al., 1983; Weekes et al., 1983; Farrell et al., 1988; Metcalf and Weekes, 1990; Grutter and Blum, 1991; Sano et al., 1991). Furthermore, irrespective of the feeding regime employed

(intensively or conventionally-fed), insulin sensitivity will decrease with age. Development of the rumen as a result of dry feed intake however will make the calf become more insulin insensitive at an earlier age. Hostettler-Allen et al. (1994) showed that insulin and glucose concentrations in intensively-fed calves eventually become elevated to the point that there is the potential for urinary spillage of blood glucose, delayed initiation of gluconeogenic processes, and reduced feed efficiency. Although such exaggerated changes in insulin and blood glucose are less likely in conventionally-fed calves, it is possible that glycosuria, delayed initiation of gluconeogenic processes, and reduced feed efficiency might also exist in these calves. Because newborn ruminants progressively become insulin insensitive with age, it is conceivable that slowing the rate at which this occurs may translate into a metabolically more efficient animal. Improving insulin's effectiveness may also improve immune modulation (i.e., enhance disease resistance) which in turn may result in greater and (or) more efficient rates of gain.

Our primary hypothesis is that insulin sensitivity declines with age in conventionally raised dairy calves and that supplemental Cr can slow this decline. To test this hypothesis, two experiments were conducted to evaluate physiological (metabolic and hormonal) responses and performance criteria (gain, intake, efficiency) of conventionally-fed dairy calves to supplemental organic Cr. Our objectives were 1) to determine if supplemental Cr would influence insulin sensitivity (i.e., effectiveness), as measured by changes in insulin and glucose concentrations following milk feedings and following intravenous infusions of glucose, 2) to determine if supplemental Cr would influence gluconeogenesis, as measured by an intravenous infusion of propionate, 3) to determine if supplemental Cr would influence

circulating blood lipids and lipolysis, as measured by changes in glucose and blood lipid concentrations following milk feedings and following an intravenous infusion of epinephrine, and 4) to determine if supplemental Cr would influence growth performance (starter intake, body weight gain, and feed efficiency).

## CHAPTER II

### REVIEW OF LITERATURE

#### Historical Background of Chromium

Dietary chromium (Cr) research began more than forty years ago. Until recently (5 to 10 years ago) the majority of research has been conducted in humans and laboratory animals, as opposed to livestock. The earliest work, conducted in rats, revealed that Cr was required for maintaining normal glucose tolerance (Schwarz and Mertz, 1959). Since then, this response has been shown in humans, mice, squirrels, monkeys, and guinea pigs (Anderson, 1987). According to Anderson (1987), hyperinsulinemia (human, rat), glycosuria (human, rat), fasting hyperglycemia (human, rat, mouse), elevated serum cholesterol and triglycerides (human, rat, mouse), impaired growth (rat, mouse, turkey), and decreased longevity (rat, mouse) are among some of the symptoms that have been attributed to a deficiency in Cr. In humans, the dietary need for a source of Cr that can be used by the body to form the GTF-complex has been firmly established. The most convincing evidence for a Cr deficiency to lead to negative consequences (i.e., impaired glucose tolerance, requirement for exogenous insulin, peripheral neuropathy, decrease in body weight) has been observed in patients on long-term total parenteral nutrition (Jeejeebhoy et al., 1977; Freund et al., 1979; Brown et al., 1986). For example, Jeejeebhoy (1977) and co-workers showed that addition of 250  $\mu\text{g/d}$  of Cr (as  $\text{CrCl}_3$ ) into infusion solutions for two wk returned glucose tolerance to normal, peripheral neuropathy disappeared, body weight (BW) returned to normal, and exogenous insulin was no longer needed.

Currently, the only established Cr recommendation set forth by the National Research Council (NRC) has been for lactating, gestating, or growing laboratory rats (0.3 ppm; NRC, 1995). Based on estimates of the Cr content of typical diets the NRC suggests a safe and adequate level of 2 ppm for the mouse, and an allowance of 0.6 ppm for the guinea pig (NRC, 1995). In adult humans, an intake of 50 to 200  $\mu\text{g}/\text{d}$  is recommended as a safe and adequate level (NRC, 1989a). For poultry, the NRC does not suggest a Cr requirement (NRC, 1994) however, based on available data it has been suggested that Cr will eventually be recommended at 3 ppm (Puls, 1990).

Among commercial livestock, the FDA only allows supplemental Cr to be added to swine diets. In contrast, Cr supplementation to livestock diets, in most foreign countries, is allowed. The NRC currently does not provide any recommended Cr level for livestock diets but does recognize that some source of Cr that can be incorporated in the GTF is needed (NRC, 1988a,b; NRC, 1989a,b; NRC, 1994; NRC, 1995; NRC, 1996). As data continue to emerge regarding Cr essentiality in livestock diets, there will be increasing pressure to approve the use of Cr in mineral supplements. Recently, the NRC (1997) published a book entitled The Role of Chromium in Animal Nutrition that covers the majority of Cr research that has been conducted in livestock animals. Although some data supports improved performance, many of the proposed benefits are less obvious, such as improved immune response, enhanced reproduction, improved recovery from stress and (or) resistance to disease, and decreased incidences of metabolic disorders. Because supplemental Cr appears to act more as a nutrient than as a pharmacological agent, it is believed that any beneficial effects may be limited to situations where an animal is clearly or

at least marginally deficient in Cr (Anderson, 1986). Progress toward establishing Cr requirements for livestock has been slow and undoubtedly will continue to be slow until simple, inexpensive methods become available to evaluate the bioavailability (i.e., absorption and retention) and biological activity (i.e., ability to potentiate insulin) of Cr in feeds and supplements. Although a variety of Cr supplements (Cr-nicotinate,  $\text{CrCl}_3$ , Cr-picolinate, chelated Cr, and high-Cr-yeast, among others) have been included in livestock diets, relatively few comparative studies, especially in ruminants, have been conducted. The lack of comparative studies also appears to be a factor that has hindered the progress toward establishing Cr requirements for livestock.

#### Biologically Active Forms of Chromium

Chromium exists in oxidation states varying from -2 to +6 (Mertz, 1969). However, its most stable state and the state most likely to persist and have metabolic activity in biological systems, is the trivalent or +3 state (Mertz, 1969; Borel and Anderson, 1984; Wallach, 1985; Kumpulainen, 1988; Ducros, 1992). As previously mentioned, it is the GTF-complex, of which trivalent Cr is an active component, that possesses biological activity. The exact structure of the GTF is not known; however, it appears to be composed of Cr, nicotinic acid, and the amino acids glycine, cysteine, and glutamic acid (Mertz et al., 1978; Toepfer et al., 1977). The mechanism(s) by which biologically active Cr improves glucose tolerance and (or) insulin effectiveness is not fully understood. However, proposed theories include more efficient binding of insulin to its receptors (Mertz and Roginski, 1963; Mertz, 1969; Anderson, 1987), increased production or membrane insertion rates of insulin

receptors (Anderson, 1986; Evans and Bowman, 1992), or possibly, a combination of the two mechanisms (Anderson, 1987).

Similar mechanistic alterations have been used to explain the onset of reduced insulin sensitivity in humans consuming diets high in fat (Kolterman et al., 1980; Marangou et al., 1986; Bonadonna et al., 1990). Because insulin insensitivity, at least in humans and some laboratory animals, can develop rapidly in response to the level of dietary fat consumed (i.e., within d depending on the percentage of energy in the diet coming from fat), it has been suggested that the change (i.e., reduced insulin sensitivity) may primarily be a response to fat intake, and that obesity only exaggerates the effect (Harris and Kor, 1992). This further suggests that tissue sensitivity to insulin is in all likelihood maintained/regulated by both acute (i.e., binding capacity of insulin receptors) and longer term (post-receptor) mechanistic changes at the cellular level.

Supplementation of both organic and inorganic trivalent Cr have been shown to elicit biological responses; although, the degree to which, and the time required before, observable biological responses are elicited, seem to vary. An understanding of why these differences occur is not readily apparent, but it is known that bioavailability (i.e., absorption and retention) and biological activity (i.e., ability to potentiate insulin) varies among the Cr compounds that have been included in diets to increase Cr intake. In processed feeds and foods the Cr that exists is not well understood; however, it is thought that most of this Cr is in a form that provides little, if any, Cr to the glucose tolerance factor complex. Inorganic sources of Cr are also thought to provide little, if any, benefit as a source of Cr for the GTF complex (Mertz et al., 1965; Anderson et al., 1983; Anderson and Kozlovsky, 1985;



Anderson, 1988) until converted to an organic form, but where or how this may occur is unclear (Borel and Anderson, 1984).

The absorption of inorganic Cr has been estimated to be between 0.4% and 3% (Dowling et al., 1989), similar to that which has been reported for Cr found inherently in feed (0.5 and 2.0%; Anderson and Kozlovsky, 1985). Organic synthetic Cr is thought to be absorbed three to fourfold more effectively than inorganic Cr (Evans, 1989b; Mertz, 1976), but may still be less than the 10 to 25% estimated absorption from the organic form found in Brewer's yeast (Mertz, 1976). More recently, humans given 400  $\mu\text{g}$  Cr as Cr picolinate/d were estimated to have absorbed only 2.5% of the Cr (Gargas et al., 1994). Current thinking is that only organic trivalent Cr will be incorporated into the GTF complex. In addition to the previously mentioned factors that have hindered estimating Cr requirements, Cr analysis is technically demanding and requires specialized instruments capable of making sophisticated background corrections. Furthermore, questions persist as to whether the total Cr content of a diet bears much, if any, relationship to its biological activity (Vobecky et al., 1979).

#### Effect of Chromium on Carbohydrate Metabolism

Impaired glucose tolerance (i.e., when glucose is cleared from the blood at a slower rate) is the classical metabolic observation resulting from a Cr deficiency (Wallach, 1985; Mertz, 1993). Although the mechanistic effect(s) of Cr, or more accurately the Cr-GTF-complex, at the cellular level (i.e., receptor) still need to be clarified, it appears that impaired (i.e., decreased) insulin responsiveness (i.e., effectiveness) may be the fundamental biological malfunction, and that all other symptoms that manifest themselves are a

consequence of this malfunction. In support of this, studies with epididymal fat tissue taken from Cr-sufficient and Cr-deficient rats showed that exogenous insulin only increased glucose uptake in tissues taken from Cr-sufficient rats (Mertz, et al., 1961; Mertz and Roginski, 1971; Mertz, 1981). Tissue samples from the Cr-sufficient rats were also able to convert more glucose to glycogen (Roginski and Mertz, 1969). In vitro, the addition of glucose and insulin failed to increase  $^{51}\text{Cr}$  binding to tissues (brain, kidney, liver) in which glucose uptake is independent of insulin, but in insulin sensitive tissues (muscle, adipose) glucose and insulin increased  $^{51}\text{Cr}$  binding (Morris et al., 1993). In hypoglycemic patients receiving supplemental Cr, insulin binding and insulin receptor numbers on red blood cells were observed to have increased (Anderson et al., 1987). Likewise, Berrio et al. (1995) showed that red blood cell ghosts and isolated adipocytes obtained from pigs fed diets containing Cr had a greater ability to bind insulin.

In humans and laboratory animals, ingestion of high (> 40% of energy) and moderate (> 30% of energy) fat diets has been shown to reduce insulin sensitivity within d or wk, respectively (Storlein et al., 1986; Sidery et al., 1990). Studies of this nature (i.e., added fat) have been interpreted to suggest that both immediate (i.e., binding to receptors) and longer term (i.e., post-receptor) mechanistic alterations are probably involved in regulating insulin sensitivity (Harris and Kor, 1992). Supplementation of Cr (as  $\text{CrCl}_3$ ) to humans considered to be hyperglycemic and hypoglycemic resulted in lower and greater glucose concentrations, respectively, at 90 min following a glucose challenge (Anderson et al., 1983). These findings suggest that Cr is probably involved in more than just increasing insulin's binding capacity to its receptors and that post-receptor changes are also occurring.

Further evidence that the mechanistic effects of Cr (i.e., GTF) are mediated through insulin action is strengthened by the observation that supplementing Cr, in the absence of insulin, did not increase glucose uptake by insulin sensitive tissues (Anderson, 1987).

**Human and Laboratory Animal Studies.** In overt-diabetic individuals displaying abnormal glucose tolerance following an oral glucose tolerance test, supplemental Cr was found to increase glucose clearance (Liu and Morris, 1978; Grant and McMullen, 1982; Polansky et al., 1982). Similarly, Elias et al. (1984) showed that giving supplemental Cr to Type-II diabetics increased insulin sensitivity in these subjects and reduced their dependence for exogenous insulin. Mildly-hyperglycemic individuals consuming diets low in Cr were observed to display low glucose tolerance; however, once subjects began receiving supplemental Cr, glucose tolerance returned to normal (Anderson et al., 1991). In this study circulating insulin concentrations also returned toward more physiological normal levels. In elderly volunteers fed a Cr-rich Brewer's yeast as opposed to a Cr-deficient Torula yeast Offenbacher and Pi-Sunyer (1980) observed that the volunteers fed the Cr-rich yeast had greater glucose tolerance and lower plasma insulin levels. Administration of supplemental Cr orally, intravenously, and via a stomach tube to rats has also been shown to lower fasting glucose concentrations and increase glucose clearance (Mertz, 1974b; Mertz, 1976; Evans, 1989a; Evans, 1991; Evans and Pouchnik, 1993). Although both, organic and inorganic forms of Cr have been shown to decrease fasting serum glucose concentrations, Vinson and Hsiao (1985) reported greater decrease's following supplementation of organic Cr.

**Livestock Studies.** After an early study showed that a synthetic, Cr-containing GTF substance was biologically active in pigs (Steele et al., 1977) there was a lag of 10 to 13

years before studies were again initiated. Since then a plethora of studies have been conducted to evaluate different Cr compounds and to establish Cr requirements. The majority of studies however have been designed to investigate whether growth performance or carcass criteria were affected, and not necessarily whether glucose homeostasis or insulin action were influenced. Nonetheless, some studies have provided evidence to suggest that supplementing diets with Cr may improve insulin action in pigs (Evock-Clover et al., 1993; Lindemann et al., 1995; Amoikon et al., 1995). Berrio et al. (1995) also showed that binding of insulin to red blood ghosts cells and isolated adipocytes was greater in samples taken from Cr-fed compared with control-fed pigs. By contrast, Ward et al. (1994) found less binding of insulin to liver cell membranes in samples taken from Cr-fed compared with control-fed pigs.

Ruminants use volatile fatty acids rather than glucose as their major energy source and their response to insulin is perhaps less well defined than that of monogastric animals. Insulin seems to have less anabolic effect on ruminant muscle and adipose tissues (Vernon and Sasaki, 1991; Lobley, 1993) and glucose uptake by the mammary cells appears to be independent of the action of insulin (Brockman, 1986; Vernon and Sasaki, 1991). Superficially, there seems little reason to anticipate that supplemental Cr would be of benefit to the ruminant. Nonetheless, impaired insulin effectiveness (i.e., non-insulin diabetic-like state) can be as severe in ruminants as in nonruminant species (Jarrett et al., 1974; Vernon and Sasaki, 1991). Insulin resistance induced in lactating cows by feeding of supplemental fat has also been shown to impair amino acid transport into the mammary gland (Palmquist and Moser, 1981). Although insulin has similar metabolic effects in nonruminant and

ruminant animals, its effects on insulin-dependent tissues in ruminants are generally less apparent (Vernon and Sasaki, 1991). It seems plausible that supplementing Cr in the diets fed to ruminants should produce metabolic changes similar to those that have been observed in nonruminants.

In ruminants, the majority of Cr-supplementation studies have been conducted with “market-transit” stressed feeder calves (Chang and Mowat, 1992; Mowat et al., 1993; Chang et al., 1995; Kegley and Spears, 1995; Kegley et al., 1997b). In these studies, Cr sources (high-Cr yeast,  $\text{CrCl}_3$ , chelated Cr), supplementation levels (ranged from 0.4 to 1 ppm), and the d on which blood variables were measured differed from study to study. Therefore, some confounding factors may exist in summarizing these studies together. Nonetheless these studies all began Cr supplementation following the calves’ arrival at the experimental site (i.e., after transit) and, overall, found a slight tendency (not always significant) for Cr-fed calves to have lower prefeeding glucose concentrations during the first month after arrival; however, over time, this effect gradually diminished. In the study conducted by Kegley and Spears (1995) postfeeding (2 h) glucose concentrations were not affected by supplemental Cr (as  $\text{CrCl}_3$ , high-Cr yeast, or Cr nicotinic acid complex). However, during an IVGTT (wk 9 of Cr supplementation) the steers receiving the Cr nicotinic acid complex tended to clear glucose faster than steers receiving the other Cr sources, but clearance rates between any one of the individual groups of Cr-fed steers did not differ from the group of control-fed steers.

When Cr, as Cr picolinate, was supplemented for 8 wk, in a corn-cottonseed hull basal diet fed to Holstein calves (steers and heifers) considered physiologically adapted, no

differences in prefeeding glucose or insulin concentrations were observed between the Cr-fed and the control-fed calves (Bunting et al., 1994). However, during an IVGTT the Cr-fed steers and heifers cleared glucose faster (40 and 27%, respectively). In addition, during an IVICT, the Cr-fed heifers cleared glucose faster (44% faster than control-fed heifers) and the Cr-fed steers tended to clear glucose faster (27% faster than control-fed bulls). The faster glucose clearance rates were interpreted to suggest that Cr had increased the sensitivity of these calves' tissues to insulin. In weaned lambs, prefeeding glucose and insulin concentrations were also not affected when supplemental Cr picolinate was fed (Fornea et al., 1994; Kitchalong et al., 1995; DePew et al., 1996; Forbes et al., 1998). Kitchalong et al. (1995) and Forbes et al. (1998) also did not observe any differences when the glucose clearance rates obtained from the IVGTT and IVICT were compared between Cr-fed and control-fed lambs. However, Kitchalong et al. (1995) did observe that Cr-fed lambs had lower glucose and greater insulin concentrations in response to the IVICT conducted 2 wk after supplementation was begun compared with what was observed after 10 wk of Cr supplementation. In growing lambs, Samsell and Spears (1989) reported that prefeeding plasma glucose and insulin concentrations were also not affected by supplemental Cr, as  $\text{CrCl}_3$ . However, they did note that Cr supplementation lowered fasting (48 h) plasma glucose concentrations in lambs fed the low-fiber diet.

Kegley and Spears (1997a) conducted an experiment to determine if Cr supplementation would affect glucose metabolism and (or) insulin effectiveness in milk-fed Holstein bull calves. Calves were obtained from a local dairy at approximately 1 wk of age and fed a commercial milk replacer without or with supplemental Cr (.4 ppm) as either  $\text{CrCl}_3$ ,

or a Cr nicotinic acid complex throughout the next 9 to 10 wk of life. Glucose concentrations before and after feeding (1 h) were not different between Cr-fed and control-fed calves on any of the individual sampling d. Because glucose concentrations increased with age the authors speculated that the rise was a reflection of increased dietary lactose intakes as milk intake was adjusted to weight gain, or was a reflection of increased insulin resistance.

### Effect of Chromium on Lipid Metabolism

Perhaps the most consistently observed metabolic effects to supplemental Cr have been changes in blood lipid concentrations. Most notably, supplemental Cr has been reported to lower circulating cholesterol and NEFA concentrations in humans and laboratory animals (Doisy et al., 1976; Liu and Morris, 1978; Anderson et al., 1988; Mertz, 1993), pigs (Page et al., 1993), as well as in ruminants (Samsell and Spears, 1989; Bunting et al., 1994; Kitchalong et al., 1995; Yang et al., 1996).

Human and Laboratory Animal Studies. In middle-aged men, Wang et al. (1989) reported that individuals fed the  $\text{CrCl}_3$  or Cr-rich Brewer's yeast had lower total cholesterol and low density lipoprotein cholesterol concentrations compared with individual fed a placebo. Similarly, Offenbacher and Pi-Sunyer (1980) reported that elderly-individuals fed a Cr-rich Brewer's yeast had lower plasma cholesterol and total lipids when compared with individuals fed a Cr-deficient *Torula* yeast for 8 wk. Chromium supplementation has also been reported to reduce total cholesterol, low density lipoprotein cholesterol, and triacylglycerol concentrations, and increase high density lipoprotein concentrations in humans considered to be mildly hypercholesteremic (Press et al., 1990; Abraham et al.,

1992). In addition, Cr supplementation of various Cr sources has been reported to result in similar reductions in cholesterol levels in healthy individuals and weight-training athletes (Press et al., 1990; Lefavi et al., 1993). In rats fed hypercholesterolemic diets, Cr supplementation has been reported to decrease total cholesterol concentrations (Staub et al., 1969). O'Flaherty and McCarty (1978) also reported that Cr-fed rats released less NEFA from adipose tissues compared with control-fed rats. In various laboratory animals, decreases in total hepatic lipid content and decreased occurrences of aortic plaques have been observed following Cr supplementation to animals fed both typical and high-cholesterol diets (Abraham et al., 1980; Abraham et al., 1982a,b; Schroeder and Balassa, 1985; Li and Stoecker, 1986).

Livestock Studies. Page et al. (1993) reported that total cholesterol concentrations were lower in pigs fed Cr picolinate than in control-fed pigs. By contrast, Evock-Clover et al. (1993) and Amoikon et al. (1995) reported that total cholesterol concentrations were greater in pigs fed Cr picolinate than in control-fed pigs. In addition, Evock-Clover et al. (1993) also reported that high-density lipoprotein cholesterol concentrations were increased in Cr-fed compared with control-fed pigs. Kitchalong et al. (1995) reported that lambs fed Cr picolinate had lower cholesterol concentrations compared with control-fed lambs after 2 wk on the experimental diet; however, this effect was no longer apparent during the 7th or 11th wk of the trial. Forbes et al. (1998) also observed that supplemental Cr did not affect cholesterol concentrations in lambs. Kegley et al. (1997a) reported that milk-fed Holstein bulls receiving supplemental Cr, as  $\text{CrCl}_3$  or a Cr-nicotinic acid compound, had similar postfeeding (3 h) cholesterol concentrations throughout the 9 to 10 wk study when



compared with control-fed bulls. Holstein steers and heifers fed corn-cottonseed hull based diets supplemented with Cr-picolinate had lower total plasma cholesterol concentrations at some individual sampling times (wk 4 and 6 for steers and calves, respectively) but overall no difference was observed (Bunting et al., 1994). In Holstein steers and heifers, Bunting et al. (1994) reported no effect of supplemental Cr picolinate on NEFA concentrations; by contrast, in yearling ewes (Forbes et al., 1998) and weaned lambs (Kitchalong et al., 1995) addition of Cr picolinate to basal diets lowered NEFA concentrations. The mechanism of action of Cr on lipid metabolism is not understood. However, Mertz (1993) suggested that the more dramatic hypolipidemic effects of organic Cr in humans may be attributable simply to increased glucose tolerance with corresponding reductions in lipolysis. Samsell and Spears (1989) hypothesized that the effects of Cr on lipogenesis were probably being influenced through the actions of insulin.

#### Chromium and the Neonate

Information regarding Cr nutrition before weaning is basically limited to determining the Cr content of neonatal food sources (i.e., breast milk, infant formula, etc.) and epidemiological comparisons of Cr status among different population groups by measuring Cr content in urine, tissue, and hair samples. From studies that have measured the Cr status of pre- and full-term infants and infants from diabetic and non-diabetic mothers, it has been determined that the Cr content of newborns is two and one-half times that of their mothers. The fetus concentrates Cr in-utero, and the Cr status of the newborn has a correlation to that of the mother's Cr nutritional state (Hambidge and Baum, 1971; Saner and Gurson, 1976; Friel et al., 1985). It has also been observed that urinary Cr concentrations increase

significantly by the 3rd d postnatally (Saner and Gurson, 1976; Saner, 1979) and that tissue Cr levels decline in later infancy (Hambidge and Baum, 1971). Although the relative importance of dietary Cr throughout the postnatal period is uncertain (Friel et al., 1985), Cr metabolism is evidently initiated early in life. The best support for this seems to come from studies with malnourished infants, where it has been observed that Cr supplementation improves glucose tolerance (Hopkins et al., 1968; Gurson and Saner, 1971).

The American Academy of Pediatrics recommends that breast milk be the sole source of nutrients for infants until about 4-6 mo of age (American Academy of Pediatrics, 1976). Friel et al. (1985) reported that the major source of Cr for infants comes from milk sources; however, milk, particularly artificial milk formulas, reportedly contain low concentrations of Cr (Hambidge, 1974). Other studies report that the Cr content of commercial milk formulas is greater than that of human breast milk (Vobecky et al., 1979; Kumpulainen and Vuori, 1980; Casey and Hambidge, 1984). Whichever the case, the Cr obtained from milk sources is considered small. As with most Cr measurements, the concentration of Cr measured in human breast milk has decreased over the past thirty years; ranging from a high of 50.5 ng/mL in 1966 to a low of 0.18 ng/mL in 1992 (Anderson et al., 1993). Similarly, the Cr content of cows' milk is also considered small and probably provides less than 0.5 ng/mL (Anderson et al., 1992). Improvements in methods of analysis and an increased awareness of Cr contamination during all phases of collection and analysis have led to increasingly accurate values for Cr in tissues and body fluids over the past decade. The older values for Cr, in hair, however, appear to still be reliable because hair

contains 200- to 1000-fold higher levels of Cr compared with those contained in tissue and body fluid samples (Anderson et al., 1993).

Anderson et al. (1993) reported that by d 60 postpartum the Cr concentration of human breast milk was 0.18 ng/mL. At this concentration, when breast milk is the sole source of Cr intake, an infant would need to consume 55.6 L in order to obtain the minimum suggested Cr intake of 10  $\mu$ g/d. Realistically, at a typical daily breast milk intake of 715 mL/d by d 60 postpartum (Borschel et al., 1986) an infant would receive less than 2% of the minimum estimated safe and adequate daily intake for infants less than 6 months of age (Anderson et al., 1993).

An adequate dietary intake of Cr appears to be of particular concern for pregnant and lactating females, because during these states there is an increased burden placed on Cr stores (Hambidge and Baum, 1971; Mahalko and Bennion, 1976). By using hair Cr concentrations as a reflection of Cr status, it has been determined that Cr concentrations are greater in primiparous than multiparous women (Hambidge and Rodgerson, 1969; Mahalko and Bennion, 1976). Mahalko and Bennion (1976) further observed that it takes more than four years between pregnancies before hair Cr concentrations return to pre-pregnancy levels. Thus it follows that an infant born within four years of its sibling would have a greater likelihood of being Cr deficient. Extrapolation of these findings to newborn ruminants would suggest that, with each successive lactation, the amount of Cr that the newborn would obtain from its dam would decrease. Research in this area is needed to determine whether Cr supplementation to the dam, and subsequently to the calf, would be beneficial.

Friel and co-workers (1985) conducted a study of 103 infants and found that at three mo of age 23% of pre-term babies and 35% of full-term babies failed to meet the minimum recommended safe and adequate daily Cr intake. As already mentioned, marginal dietary intakes of Cr have been related to impaired glucose tolerance in some human population groups. Friel and co-workers (1985) suggested that abnormalities in carbohydrate metabolism, not infrequent in early post-natal life, may be related to sub-optimal Cr status.

Saner et al. (1980) conducted a study with newborn infants in which an intravenous glucose tolerance test was performed before 24 h of age, then gave Cr ( $250 \mu\text{g CrCl}_3$  in 2 ml of tea), and then repeated the test approximately 13 h after the administration of Cr. An average glucose clearance rate of 1.22% per min was observed (1.34 and .90% per min for the Cr-supplemented and control group, respectively) among the infants when they were less than 24 h old and an average clearance rate of 2.67% per min was observed (2.58 and 2.04% per min for the Cr-supplemented and control group, respectively) among the infants when they were about 37 h old. A significant increase in glucose clearance was observed on the 2nd d of life in both groups of newborns. These authors concluded that the low glucose clearance rate in the newborn, irrespective of administered Cr, may be taken as evidence that the active role of Cr may be inadequate in the newborn. The lack of precise control of glucose homeostasis in the human neonate has been postulated to be a secondary consequence of either a decreased sensitivity to or a decreased resistance to insulin (Cowett et al., 1983).

In adult monogastric animals, blood glucose and plasma insulin generally increase following oral or intravenous glucose tolerance tests. In response to an influx of glucose

into the blood stream, the body responds by inhibiting hepatic glucose production and increasing the rate at which glucose is taken up by tissues, thereby avoiding the development of hyperglycemia (Unger, 1981). Regulation of glucose homeostasis in newborns however, may be less well developed, since evidence has shown that hepatic glucose production is not normally suppressed following intravenous glucose tolerance tests in infants (Cowett et al., 1983), dogs (Varma et al., 1973), and rats (Ferre et al., 1985; Issad et al., 1987). Studies that have employed intravenous glucose tolerance tests to measure glucose homeostasis in newborn infants (<48 h) have shown that low glucose clearance rates characterize the 1st few d of life (Baird and Farquhar, 1962; Bowie et al., 1963; Falorni et al., 1974). A delay in pancreatic insulin release has been suggested as a primary reason for the slowed rate of glucose clearance (Saner et al., 1980). Insulin insensitivity appears to be a common characteristic of newborns; however, the persistency of this insulin insensitivity also appears to vary among species. In human newborns, insulin sensitivity increases within the 1st wk of life (Baird and Farquhar, 1962; Bowie et al., 1963; Falorni et al., 1974), in rats between the 2nd and 4th wk of life (Issad et al., 1987), and in dogs between the 7th and 8th wk of life (Varma et al., 1973). Although, the mechanism(s) responsible for these changes are unclear, it appears that a nutritional component may exist. For example, in the rat experiment, an increase in insulin sensitivity coincided with the time that the pups were weaned from a high-fat, low-carbohydrate diet (milk) to a high-carbohydrate, low-fat diet (laboratory weaning diet; Issad et al., 1987). In this study, it was suggested that changes in insulin sensitivity (in this case an increase) may be, to a degree, influenced by nutrition (i.e., high fat vs. high carbohydrate diet) but that there was also a possible influence of

developmental state. Whether changes in insulin sensitivity are due to nutrition, stage of development (i.e., age), or a combination of the two, in the aforementioned monogastric species, insulin sensitivity increases during the neonatal period.

Following consumption of a meal, glucose is absorbed from the intestine similarly in young ruminant (pre-weaned) and monogastric animals. Subsequently, glucose and shortly thereafter insulin concentrations rise in the blood. However, as far as glucose utilization is concerned, the tissues of the young monogastric have a greater response to insulin, as they can convert glucose to fatty acid at a high rate (this process provides a sink for glucose disposal), whereas in the ruminant this capacity is very limited (Vernon and Sasaki, 1991). In addition, the capacity to transport glucose across the plasma membrane of adipocytes is less in ruminants compared with nonruminants. Since, postprandial insulin and glucose concentrations in intensively-fed calves have been suggested to become elevated to the point that urinary spillage of blood glucose is possible (Hostettler-Allen et al., 1994). It would seem that if the tissues of intensively-fed calves could be made more responsive to insulin, glucose might potentially be used more efficiently.

To investigate this question, Kegley et al. (1997a) conducted a study with milk-fed dairy calves from 1 to 10 wk of age to determine if the insulin potentiating action of Cr would increase or at least maintain insulin sensitivity as the calves became older. Chromium was supplemented in a commercial milk replacer at 0.4 ppm as either  $\text{CrCl}_3$  or a Cr-nicotinic acid complex. Chromium did not affect growth performance, urea-N, glucose before or after (1 h) feeding, and cholesterol (3 h after feeding) on any of the sampling d. Chromium also did not affect glucose and insulin response patterns or glucose clearance rates in

response to an IVGTT or following consumption of milk replacer. However, based on the glucose and insulin changes observed following an insulin injection, these authors concluded that the tissues in the Cr-fed calves might have been more sensitive to insulin. Other observations from this experiment, but reported in a separate paper (Kegley et al., 1996) discussed the effects that supplemental Cr had on the immune system. Although most responses were minimal, the calves fed the Cr-nicotinic acid complex supplemented-replacer were observed to have a greater inflammatory response to an intradermal injection of phytohemagglutinin injection than the control-replacer-fed calves. Serum cortisol concentrations following a challenge with an infectious bovine rhinotracheitis virus were also lower in Cr-fed compared with control-fed calves. From this study, these authors concluded that addition of Cr to milk replacer may enhance immunity and, thus, increase disease resistance in young calves.

The only other study conducted with ruminants in which the animals might be able to be classified as neonates was conducted by Arthington et al. (1997). In this study, 3 mg Cr/d of a high-Cr-yeast product was supplemented to a calf starter and fed to weaned (6 to 8 wk old) Holstein bull calves over the next 53 d. Supplemental Cr did not affect ADG or rectal temperatures. On d 53 of the trial, calves were inoculated with bovine herpesvirus-1 and 24 h later blood and urine samples and rectal temperatures were collected at 6-h intervals over the next 6 d. Cortisol, ACTH, plasma tumor necrosis factor- $\alpha$ , rectal temperatures, and trace mineral excreted in the urine were not affected by Cr. These authors concluded that Cr supplementation using high-Cr-yeast did not alter stress responses of young, weaned calves experimentally inoculated with bovine herpesvirus-1.

### Implications of Chromium During Stress

Stress, in general, is the body's response to a threat or demand (i.e., some stimuli) arising from a new or changing situation where the bodies' reaction to that stimulus disturbs its normal physiological equilibrium and (or) homeostasis (Hadley, 1988; Guyton, 1991). Two stages of stress exist. During the initial stage, the alarm phase, the body mobilizes its fight or flight defenses, either to resist the stress-causing factor or to adapt to it. In this stage, the pituitary-adrenocortical system pours hormones into the bloodstream. The pulse quickens, the lungs take in more oxygen to fuel the muscles, blood sugar increases to supply added energy, and digestion slows. In stage two, the resistance stage, the body begins to repair the incidental damage caused during the alarm stage. If the stressful situation is resolved, the symptoms brought on by the stress will gradually subside. However, if the stressful situation is not resolved vital organs can become affected which in turn may lead to an increased risk of disease or even death (Hadley, 1988; Guyton, 1991).

The initial hormonal response to stress is the release of epinephrine and norepinephrine. Although these hormones cause short-lived effects, only 20 to 30 s, they increase gluconeogenesis and glycogenolysis in the liver and skeletal muscle, increase lipolytic activity, and increase general metabolism (Hadley, 1988; Guyton, 1991). A variety of nonspecific stimuli can cause marked increases in the rate of cortisol secretion by the adrenal cortex (Hadley et al., 1988; Guyton, 1991). Cortisol, a glucocorticoid, promotes gluconeogenesis but reduces glucose utilization (Guyton, 1991). The increased rate of gluconeogenesis coupled with a reduction in the rate of glucose uptake by cells causes blood glucose concentrations to rise. Although cortisol provides a positive benefit by suppressing



the inflammatory response in the short-term (Guyton, 1991), elevated levels over prolonged periods can impair immune response, such that relatively innocuous infections can escalate to the point of extreme morbidity (Goodman, 1988). During prolonged stress, fuels that could be used to promote growth are no longer available, because they must now be used to fight off an infection or repair damaged tissue.

Evidence from studies with humans and mice suggest that various physiological or metabolic challenges (i.e., elevated intakes of glucose, low protein diets, infection, strenuous exercise, trauma) increase urinary excretion of Cr (Pekarek et al., 1975; Borel et al., 1984; Anderson et al., 1988). These findings have led some investigators to suggest that animals must be under some form of stress before a beneficial response from Cr supplementation will be observed (Anderson, 1994b; Mowat, 1994). In livestock, stress has been clearly shown to lead to reduced performance (Khansari et al., 1990).

Urinary Cr levels are recognized as an indicator of Cr mobilization, because they increase with the intensity and duration of the stress (Anderson et al., 1990). The IVGTT is the most commonly used diagnostic technique to measure the effects of Cr supplementation on insulin effectiveness. Although glucose loading increases Cr mobilization, as demonstrated by greater urinary Cr excretion, it is not nearly as great as what is observed following physical trauma or strenuous exercise. Therefore, the amount of Cr lost in the urine may be dependent on or be an indication of the degree of stress imposed (Anderson, 1994b).

Studies involving Cr supplementation to livestock that are under significant stress are limited. Researchers have reported that stressed feeder calves (stress was transportation

to a new facility) that received supplemental Cr had improved performance, reduced morbidity, and improved humoral immune function (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Mowat et al., 1993; Wright et al., 1994; Kegley and Spears, 1995). In contrast, when non-stressed feeder calves were studied over longer feeding periods, growth performance was not influenced by Cr supplementation (Chang and Mowat, 1992; Chang et al., 1995; Bunting et al., 1994, Mathison and Engstrom, 1995).

Some studies with stressed cattle have reported a decrease in serum cortisol concentrations in Cr supplemented animals (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993). Supplementation with 0, .2, .5, and 1 ppm Cr from a high-Cr yeast to transit-stressed feeder calves resulted in a linear decrease in serum cortisol concentrations during the 1st 28 d of the experiment (Moonsie-Shageer and Mowat, 1993). Chang and Mowat (1992) also reported that high-Cr yeast decreased serum cortisol concentrations in transit-stressed steers after 64 d of supplementation. By contrast supplementation of a high-Cr yeast product to younger (6 to 8 wk old) weaned calves did not affect cortisol concentrations.

Physical, nutritional, and emotional stress are all possibilities in the neonatal calf. Situations that may lead to chronic stress and consequently to elevated cortisol levels in the young calf could include extremes in temperature (hot to cold), nutrition (transition from liquid to dry fed diet), disease, surgery, restraint and almost any type of trauma. Cortisol decreases immune system functions (Kelley, 1988; Muneer et al., 1988).

### Implications of Chromium on Immune Function

In laboratory animals, supplemental Cr has been shown to increase resistance to pulmonary infections (Schroeder et al., 1964) and decrease mortality rates associated with experimentally induced acute hemorrhages (Mertz and Roginski, 1969). In feedlot cattle supplemental Cr was shown to reduce haptoglobin (an acute phase protein that increases during trauma or inflammation) concentrations (Wright et al., 1995) and increase humoral immune response and reduce morbidity (Change and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Mowat et al., 1993; Wright et al., 1995). In newly arrived transit-stressed calves, supplemental Cr reduced the incidence of bovine respiratory disease (Mowat et al., 1993; Lindell et al., 1994). Supplemental Cr has also been reported to decrease morbidity (and) or reduce the number of relapses in newly arrived feeder steers in some studies (Moonsie-Shageer and Mowat, 1993; Mowat et al., 1993; Wright et al., 1994), but not other studies (Chang and Mowat, 1992; Chang et al., 1995).

Dairy cows fed supplemental Cr during the periparturient period (6 wk pre to 16 wk postpartum) had increased in vitro concanavalin A stimulated lymphocyte blastogenic responses and prevented a decrease in blastogenic response that were observed in controls at two wk prepartum (Burton et al., 1994). Chromium supplementation with high-Cr yeast did not alter the stress responses of calves experimentally inoculated with bovine herpesvirus-1 (Arthington et al., 1997).

Kegley et al. (1996) reported that a greater inflammatory response to intradermal phytohemagglutinin (used to evaluate cell-mediated immune response) was observed in milk-fed calves receiving supplemental Cr as either a Cr-nicotinic acid complex or  $\text{CrCl}_3$ .

In these same calves, it was also observed that following an infectious bovine rhinotracheitis virus challenge, the calves that received the Cr-nicotinic acid complex tended to have lower body temperatures compared with the CrCl<sub>3</sub>-fed and control-fed calves. Serum cortisol concentrations were also lower in the Cr-fed compared with the control-fed calves. Lymphocyte blastogenic response to phytohemagglutinin and pokeweed mitogen were not affected by Cr. These authors concluded that Cr supplementation may enhance immunity and, thus, increase disease resistance in young calves. Specific high affinity insulin receptors have been found on bovine peripheral blood mononuclear cells (Mcbride et al., 1989) and the functional capacity of cultured bovine peripheral blood mononuclear cells has been found to be affected by insulin (Burton et al, 1993). Some studies, but not others, and some immunological measurements, but not all, suggest that Cr is probably important in immune modulation. Speculation would seem to suggest that the action of Cr on immune responses are similar to what has been suggested for its effect on carbohydrate and lipid metabolism. That is by improving the insulin sensitivity of immune cells. Glucose uptake by immune cells is insulin dependent. Therefore improving insulin's effectiveness may ensure that energy (i.e., glucose) is available so that the immune cells can carry-out their required functions. It is also known that as insulin sensitivity decreases, such as with a diabetic individual, immune responsiveness also decreases (Mangrum and Bakris, 1997).

#### **Chromium Effects on Growth Performance**

Since the early sixties, growth performance (i.e., rate of gain, feed intake, feed efficiency) has been measured in laboratory animals fed a variety of diets supplemented with inorganic and organic Cr sources. Supplemental Cr to typical laboratory rat and mice diets

have been reported to increase rates of gain (Schroeder et al., 1963a,b; Schroeder, 1965; Schroeder 1966; Mertz and Roginski, 1969). In addition, Schroeder (1965) compared growth curves of Cr-fed and control-fed rats and mice and concluded that the largest increases seemed to occur around weaning. In studies that have investigated whether there was an interaction between supplemental Cr and sex, most reported no effect of sex (Schroeder et al., 1963b; Schroeder, 1965; Schroeder 1966), although one study (Mertz and Roginski, 1969) reported the supplemental Cr enhanced the growth rate of females to a greater degree than that of males. Two more recent reports failed to find any beneficial improvements on performance in rats fed supplemental Cr (Hasten, 1994; Bush, 1997). Why some studies conducted with laboratory animals have reported that supplemental Cr improves some performance criteria and other similarly conducted studies have failed to find a difference is not readily apparent. However, one theory that has been suggested is that these inconsistencies may be because of an individual animal's Cr status, prior to the time supplementation was begun. That is, animals that are already adequate in Cr may not show a response to supplemental Cr. Mooradian and Morely (1978) suggested that improvements from Cr supplementation will be most likely to take place only in Cr-deficient subjects. In addition, the use of different Cr sources and the bioavailability of those sources may also be a contributing factor to the lack of consistency between studies.

Chromium effect on performance criteria have also been variable in studies conducted with livestock. As suggested for the laboratory animals, initial Cr status may be a contributing factor. Furthermore, formulating Cr-free diets for livestock is less practical than for laboratory animals. Therefore it is even more difficult to ensure that the only

source of Cr that the animal receives is that which is part of the treatment. Possibly as a result of these limitations, Cr supplementation studies conducted with livestock have been shown to either result in slight increases (Chang and Mowat, 1992; Page et al., 1993; Chang et al., 1995), or have no effect (Bunting et al., 1994; Chang and Mowat, 1992; Evock-Clover et al., 1993; Kitchalong et al., 1995; Lindemann et al., 1995; Page et al., 1993; Chang et al., 1995) on growth performance.

In pigs, some studies have reported greater growth rates in Cr-fed compared with control-fed (Page et al., 1993; Van Heugten and Spears, 1994) while others have reported no effect of supplemental Cr (Evock-Clover et al., 1993; Ward et al., 1994). In newly arrived market-transit calves, Chang and Mowat (1992) reported that addition of .4 ppm Cr as a high-Cr yeast to the ration improved ADG (average daily gain) by 30% and feed efficiency by 27% during the 1<sup>st</sup> 28 d after arrival in the Cr-fed calves compared with control-fed calves that did not receive an oxytetracycline injection, but not compared with the control-fed calves that received an oxytetracycline injection within 48 h after arrival. Moonsie-Shageer and Mowat (1993) showed a linear relationship between Cr intakes (0 to 1 ppm) and growth performance responses during the initial 28 d after arrival of transit-stressed feeder steers. However, in this study, performance responses were again similar after calves were switched from receiving to finishing rations. In stressed feeder calves, supplementation of Cr at .5 ppm to the ration as an amino acid-Cr chelate has also been reported to improve ADG (Mowat et al., 1993). However, in this study, growth improvements were attributed to fewer morbid calves in the Cr supplemented group.

Supplementation of .37 ppm Cr as chromium picolinate to nonstressed calves fed corn-cottonseed hull basal diets over a 56-d period did not affect ADG, DMI, or feed efficiency (Bunting et al., 1994). Likewise, no differences were observed on gain, intake, or efficiency when supplementing .2 ppm Cr from high Cr yeast to growing (70 d) and finishing (68 d) cattle (Chang et al., 1992). In lambs receiving high concentrate rations, addition of Cr as Cr picolinate was also reported to have no effect on ADG or feed intake (Kitchalong et al., 1995). In calves fed milk alone through the initial 9-wk of life, supplementation with Cr as  $\text{CrCl}_3$  or a Cr nicotinic acid complex did not affect rate of gain or feed efficiency compared with control-fed calves (Kegley et al., 1997a).

Most reports on changes in body composition in response to addition of supplemental Cr to rations have been reported in studies conducted with pigs, as opposed to ruminants. In pigs the more commonly observed effects of Cr supplementation on carcass changes seem to be increased longissimus muscle area and decreased tenth rib fat thickness (Page et al., 1993; Smith et al., 1994; Lindemann et al., 1995). In sheep, Kitchalong et al. (1995) also reported that Cr picolinate reduced tenth rib fat thickness and also showed there was a tendency for a decrease in pelvic fat. In cattle, Chang et al. (1992) and Mathison and Engstrom (1995) reported that Cr supplementation did not affect any of the carcass measurements taken; however, Claeys et al. (1994) did report higher quality grades for Cr-fed compared with control-fed steers.

#### Chromium Effects on Milk Production

Glucose uptake by the mammary cells appears to be independent of the action of insulin (Brockman, 1986). Therefore, it would appear that there would be little benefit from

Cr supplementation on milk production. However, emerging data from lactation studies suggests that supplemental Cr may increase milk yield during early lactation (Yang et al., 1996, Besong et al., 1996). Although it is not clear how supplemental Cr may increase milk yield in early lactation, one possibility is that a slight reduction in the rate of mobilization of fatty acids from adipose tissue may simply help stabilize hepatic fat metabolism, reduce hepatic ketogenesis, and perhaps allow feed intake to increase more rapidly after calving. However, only in the Besong et al. (1996) study was the increase in milk yield accompanied by increased feed intake. Yang and co-workers (1996) postulated that increased milk yield may be the result of the indirect effects of Cr on gluconeogenesis. Subiyatno et al. (1996) in early lactation heifers and Sano et al. (1996) in stressed rams showed that, following an i.v. propionate loading test, the conversion of propionate to glucose increased in those animals receiving supplemental Cr. This suggests that Cr may, by some yet unknown mechanism, increase gluconeogenesis. A more direct hypothesis for the increase in milk production might be that Cr promoted the activity of the insulin-like growth factor (IGF) receptors, because IGF receptors have a structural and functional homology similar to that of insulin receptors (Yang et al., 1996). Subiyatno and co-workers (1996) showed trends for increased circulating IGF-1 in Cr supplemented, early lactation cows following a propionate challenge.



# CHAPTER III

## PERFORMANCE AND METABOLIC RESPONSES OF PRE- AND POST-WEANING HOLSTEIN CALVES SUPPLEMENTED WITH CHROMIUM PICOLINATE

### Introduction

Chromium is well established as an essential trace element for man and laboratory animals (Anderson, 1987; Mertz, 1993). Chromium appears to be an integral component of a molecular complex known as the glucose tolerance factor which facilitates the cellular binding and action of insulin. There is increasing evidence that Cr may be a required nutrient in livestock diets. Supplemental Cr has improved glucose tolerance and has improved a number of production parameters in swine (Page et al., 1993; Amoikon et al., 1995). In ruminants, positive production responses to supplemental Cr seem to depend upon the presence of stressors, such as "market-transit" stress (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Mowat et al., 1993) or early lactation (Subiyatno et al., 1996). Supplemental Cr has had only modest effects on glucose tolerance or other indices of carbohydrate metabolism in functional ruminants (Bunting et al., 1994; Kegley and Spears, 1995; Kitchalong et al., 1995; Subiyatno et al., 1996). However, functional ruminants derive little glucose from intestinal absorption and the role of insulin in these animals is perhaps less well defined than in the nonruminant (Brockman, 1986).

Because glucose is absorbed in comparatively large quantities in the milk-fed ruminant, the importance of insulin to glucose homeostasis is analogous to that of the nonruminant. In addition, there is evidence that milk-fed calves become insulin resistant with age, resulting in exaggerated postfeeding elevations in insulin and glucose in the blood

(Hostettler-Allen et al., 1994). Under these conditions, there is the potential for urinary spillage of blood glucose and reduced efficiency. In addition, Grutter and Blum (1991) reported that insulin secretory mechanisms may not be fully developed in young calves. Therefore, it seems plausible that neonatal calves would be more metabolically responsive to Cr than adult cattle. The limited data that are available with milk-fed calves were not conclusive regarding the effects of supplemental Cr on carbohydrate metabolism (Kegley et al., 1997a). The study described herein was conducted to more clearly delineate the effects of supplemental Cr on carbohydrate metabolism in conventionally-fed calves from birth through weaning.

### **Materials and Methods**

**Animals and Feeding.** Forty-two Holstein calves born at the campus dairy farm in Baton Rouge, were used in an experiment approved by the Institutional Animal Care and Use Committee of the LSU Agricultural Center. Although the calves were born over a five-month period (August 23 to December 5, 1994), the experimental period for each calf lasted from 4 d to around 8 wk ( $d\ 53 \pm 3$ ) of age. Calves were removed from their dams within 24-h of birth, weighed, and individually housed in 2.5-m<sup>2</sup> hutches with a 2.3-m<sup>2</sup> wire enclosure attached to the hutch. During the first 3 d after birth, calves received colostrum only (1.9 liters twice daily) and were trained to drink from buckets. Any colostrum not voluntarily consumed was fed by bottle or esophageal feeding tube. Calves readily drinking from buckets and showing no apparent health problems at 3 d of age were divided into sex and birth date peer groups, and then randomly assigned to treatment (11 male and 10 female calves per treatment). Treatment consisted of diets either without (control) or with 1 ppm

supplemental chromium (Cr). Chromium was supplied as chromium picolinate (CrP; 12% Cr; Nutrition 21, San Diego, CA).

Beginning on d 4 and until 5 wk of age (weaning), calves received a commercial milk replacer with a guaranteed analysis of 22% CP and 15% fat (Nurstrate®, Moorman's, Inc, Quincy, IL). Each calf received 250 g of DM as milk replacer in 1600 mL of  $40 \pm 1^\circ\text{C}$  tap water at 0800 and 1600 daily until weaning. Chromium picolinate is poorly soluble in water, hence, to ensure complete consumption, the entire daily allotment was suspended in 15% of the evening feeding and given via a bottle until calves were weaned. On d 4 and 5 of age, Cr-supplemented calves received a priming dose of 21 mg of Cr daily and from 6 d of age until weaning they received 4.2 mg/d of Cr. After calves were weaned CrP, in a dextrose carrier, was supplemented in the starter.

On the morning following collection of wk 2 blood samples calves were offered a commercial calf starter with a guaranteed analysis of at least 16% CP and 2% fat (Purina® commercial calf starter/grower (coarse) BVT 60; Purina Mills, Inc, St. Louis, MO). Starter intake was recorded and fresh feed offered at each feeding. When a calf had consumed all of its starter between feedings, an additional 100 g of starter was offered. All calves were weaned at around 5 wk of age at an average starter intake of .96 kg/d.

Sample Collection and Metabolic Challenges. Body weight (BW) measurements were taken shortly after birth and at noon when calves were around 2, 4, and 8 wk old. At 1, 2, 3, 4 and 5 wk of age, blood was collected immediately before (prefeeding) and 2 h after (postfeeding) the evening meal by jugular venipuncture into 7-mL evacuated tubes

containing potassium oxalate and sodium fluoride (Sherwood Medical, St. Louis, MO). Postfeeding blood sampling was discontinued for the last eight calves added to the study.

Metabolic challenges were conducted on half the calves. An i.v. glucose tolerance test (IVGTT) was conducted at the end of the 2nd wk of life (the d prior to starter access) and during the 8th wk of life to measure the calves abilities to clear a glucose load. A propionate loading test (PLT) was also conducted on the same calves during the 8th wk of life to measure the effects of Cr supplementation on gluconeogenic potential. The IVGTT was performed 4 h after the morning milk feeding at 2 wk of age and after an overnight fast (16 h) at 8 wk of age. Calves were fitted with 14-G jugular catheters (Quik-Cath®; Baxter Healthcare, Deerfield, IL) and were allowed at least 1 h of rest prior to the IVGTT. The IVGTT consisted of infusing a glucose solution (500 mg/kg of BW in a 50% w/v solution in sterile saline) as an i.v. bolus dose over a 30- to 60-s interval. Prior to infusion of glucose, one blood sample was drawn (0 min) and then glucose was administered (1 mL/kg BW) i.v. through the catheter. Blood was then drawn at 10, 15, 20, 30, 40, 50 and 60 min relative to glucose infusion. Following completion of the IVGTT conducted at 8 wk of age, calves were given access to water only during a 2-h rest period prior to beginning the PLT. The PLT consisted of a pulse i.v. infusion of propionate (3 mmol of Na-propionate/kg BW as a 1.84 M solution in sterile saline) over a 2-min interval. Blood samples were collected prior to and 5, 10, 15, 20, 40, 60, 90, and 120 min relative to propionate infusion. To ensure that none of the infusion solutions remained in the catheter sheath, following infusion of glucose or Na-propionate, sterile saline (5 mL) was flushed through the catheter. Catheter patency was maintained with 6% sodium citrate in sterile saline.

**Blood Processing and Analyses.** Blood samples were placed in ice immediately after withdrawal and were centrifuged within 1 h at 3,000 x g for 10 min at 4°C to obtain plasma. Plasma was harvested and stored at -15°C. Weekly blood samples were analyzed for plasma glucose, insulin, and nonesterified fatty acids (NEFA) concentrations in the 0 and 2 h samples, whereas plasma cortisol and IGF-1 concentrations were determined only in the prefeeding samples. Commercial kits were used in the colorimetric determination of plasma glucose (Sigma Tech. Bull. No. 315; Sigma Chemical, St. Louis, MO) and NEFA (NEFA-C Kit; Wako Chemicals USA, Richmond, VA.). Plasma cortisol was determined by a commercial kit (Cortisol RIA Kit No.DSL-2000; Diagnostic Systems Laboratories Inc., Webster, TX.). Plasma insulin and IGF-1 were determined by RIA as described by Sticker et al. (1995). For blood samples collected during the PLT, propionate was precipitated as a Na salt from deproteinized plasma as described by Ryan (1980). The propionate salt was resuspended in water and subjected to standard gas chromatography (Shimadzu GC-17A; Shimadzu Scientific Instruments, Inc., Columbia, MD) with samples corrected to 100% recovery using 2-ethybutyric acid as an internal standard.

**Statistical Methods and Calculations.** Glucose and propionate clearance rates were computed for each calf following the IVGTT or PLT, respectively (Kaneko, 1989). Intervals used to compute glucose clearance rate were 15 to 30 min and 10 to 40 min for the IVGTT and PLT, respectively. The interval to compute propionate clearance rate was 5 to 20 min for the PLT. Changes in plasma glucose in response to the IVGTT and PLT were further evaluated by computing area under the response curves (AUC) relative to basal levels using trapezoidal geometry.

Data for initial BW, starter intake, and kinetic analysis of metabolite challenges were analyzed by least squares ANOVA and PROC GLM of SAS (SAS®, 1989). The model was a factorial including treatment, sex and treatment by sex interaction. Analysis of final and intermediate BW measurements and feed efficiency included birth weight as a covariable. Data for prefeeding and postfeeding hormone and metabolite concentrations were analyzed as a split-plot. In the whole plot, treatment, sex and treatment by sex were tested against calf (treatment x sex) means square. In the sub-plot, calf age (wk) and all interactions of age with treatment and sex were tested against residual mean square. The PROC UNIVARIATE procedure of SAS (SAS®, 1989) was used to test for normality and a Shapiro-Wilks test value of 0.1 was selected for rejection of the null hypothesis. When normality was rejected, data transformation was used to achieve normality and transformed values analyzed. Means are reported in original units and all statistical analyses were conducted using the GLM procedure of SAS (SAS®, 1989). Aside from the previously mentioned blood samples that were not collected there was one CrP-fed heifer in which only performance data was collected and one CrP-fed bull in which only its weekly blood variable measurements and performance data through wk 2 were used.

### **Results and Discussion**

**Performance.** Twenty-two male and twenty female Holstein calves entered the trial at 4 d of age. Aside from minor, controllable bouts of scours that occurred primarily before weaning, calves were healthy throughout the trial. Calves averaged  $38.5 \pm 4$  kg at birth and gained an average of  $27.8 \pm 6.5$  kg over the 8 wk trial. As expected, rate of weight gain was influenced by the age of the calf ( $P = .0001$ ). Average daily gain was lowest while

calves were fed milk only ( $.17 \pm .13$  kg/d/calf) and increased exponentially ( $r^2 = 1$ ) over the ensuing 2 to 3-wk weighing intervals. Over the initial 2-wk interval that calves had access to starter, calves gained an average of  $.36 \pm .16$  kg/d, and over the final 2- to 3-wk interval calves gained an average of  $.77 \pm .13$  kg/d. Over the entire trial, calves gained an average of  $.50 \pm .1$  kg/d and  $.47 \pm .05$  kg of BW was gained per kg of DM intake. Starter intake was minimal (less than  $.22$  kg/d) between 2 and 3 wk of age, then steadily increased ( $P = .0001$ ) throughout the remainder of the experiment. Based on three-day-averages, starter intakes (as-fed) of  $.45$ ,  $.68$ ,  $.91$ ,  $1.36$ , and  $1.84$  kg/d were reached at  $28 \pm 4$ ,  $32 \pm 4$ ,  $35 \pm 4$ ,  $39 \pm 4$ , and  $44 \pm 5$  d of age, respectively.

In calves, rate of gain characteristically increases exponentially throughout the first several months of life (Roy, 1980). Reported ADG can also be influenced by differences in feeding regimes and differences in the time frames used to report daily gains. These inherent and imposed differences between studies make comparing rates of gain from one study to the next difficult. Through the first 90 d of life, a desirable rate of gain for dairy herd replacements has been suggested to be about  $.875\%$  of birth weight daily or about  $.42$  kg/d (Roy, 1980). This is about  $.08$  kg/d less than observed in the current trial. Holstein calves fed milk alone and milk plus grain and (or) hay over the initial 28 d of life have been reported to gain  $.26$  kg/d and  $.53$  kg/d, respectively (Quigley et al., 1991). Estimates of growth rates in Holstein calves during the first several months of life have ranged from  $.39$  to  $.66$  kg/d across a variety of feeding regimes (Doppenberg and Palmquist, 1991; Quigley et al., 1991; Quigley and Bernard, 1992; Kegley et al., 1997a).

The effects of Cr supplementation and sex on rate of gain were analyzed over three intervals: 1) milk-fed only, 2) starter plus milk, and 3) over the entire trial. For all the intervals investigated ADG did not differ ( $P > .10$ ) between calves fed control or Cr-supplemented milk replacers or between bulls and heifers (Table 3.1). However, when calves received milk alone, there was a tendency for bulls given supplemental Cr to gain more weight and for heifers given supplemental Cr to gain less weight compared to control-fed calves (treatment x sex;  $P = .15$ ; Table 3.1). This tendency was not evident once calves began receiving starter (treatment x sex;  $P = .27$ ); however, over the entire trial, bulls given supplemental Cr gained more weight and heifers gained less weight than control calves (treatment x sex;  $P = .10$ ; Table 3.1). Kegley et al. (1997a) reported that supplementation with either organic or inorganic Cr had no effect on rate of gain or feed efficiency in Holstein bull calves fed milk alone during the first 9 to 10 wk of life. In older calves, supplemental Cr has been reported to improve performance in some studies (Moonsie-Shageer and Mowat, 1993; Mowat et al., 1993) but not others (Bunting et al., 1994; Kegley and Spears, 1995). Starter intakes did not differ between treatments or sexes ( $P > .10$ ; Table 3.1). When calves were fed only milk, all calves received the same amount of DM daily. It is possible that, because bulls were slightly ( $P = .17$ ; Table 3.1) larger than heifers at birth, bulls may have been more nutrient deficient than were the heifers.

**Plasma Metabolites and Hormones.** Regardless of calf age, sex did not affect ( $P > .10$ ) pre- and postfeeding blood metabolite concentrations; hence, only the effects of dietary treatment and age are presented in Tables 3.2 and 3.4. With the exception of prefeeding NEFA (below), there were no interactions ( $P > .10$ ) between treatment and age for any of



Table 3.1. Least squares means for BW gain and starter intake in calves supplemented with chromium picolinate.

Item	Treatment				SEM	P > F <sup>1</sup>		
	Control		Chromium			Treatment	Sex	Treatment x Sex
	Bulls	Heifers	Bulls	Heifers				
n <sup>2</sup>	11	10	11	10				
Initial BW, kg	40.5	36.9	38.5	38.2	1.36	.83	.17	.22
BW gain, kg/d								
week 0 to 2 <sup>3</sup>	.15	.21	.18	.12	.04	.40	.99	.15
week 0 to 8	.47	.53	.53	.48	.03	.76	.94	.10
week 2 to 8 <sup>4</sup>	.60	.63	.66	.62	.04	.52	.90	.27
Starter intake, kg of DM/d (week 2 to 8)	.92	1.04	1.09	1.02	.08	.36	.75	.25

<sup>1</sup>Probability of greater F-value.

<sup>2</sup>For one bull calf in the CrP treatment only its initial and week 2 BW data were used.

<sup>3</sup>Period during which calves received only milk.

<sup>4</sup>Period during which calves received milk plus grain (week 2 to 5) and grain only (week 5 to 8).

the prefeeding or feeding-response plasma metabolite concentrations measured in this experiment (Tables 3.2 and 3.4). Generally, the plasma glucose response to feeding increased until calves were about 3 wk of age and then declined to wk 5 of age (age effect;  $P = .003$ ; Table 3.2). This age-related pattern was more obvious in Cr-supplemented calves; however, glucose responses in calves fed the control milk replacer were relatively consistent from one wk to the next (treatment  $\times$  age trend;  $P = .12$ ; Table 3.2). The physiological relevance of this observation relative to the effect of Cr on insulin function is not clear. Chromium had no independent effects ( $P > .10$ ; Table 3.2) on pre- or postfeeding concentrations of glucose or insulin. When intensively-fed veal calves consumed a normal meal, plasma glucose concentrations have been observed to rise above the assumed renal threshold for urinary glucose excretion in calves (Hostettler-Allen et al., 1994). The renal threshold for calves has been estimated at between about 8 and 10 mM (Wijayasinghe et al., 1984; Scholz and Hoppe, 1987; Hostettler-Allen et al., 1994). Hostettler-Allen et al. (1994) concluded that urinary glucose loss was probably only a transient phenomenon, but represented a potential source of lost energy that could contribute to reduced feed utilization and growth performance; however, in their study, ADG and gain:feed ratio by calves was not visibly diminished. In our study, plasma glucose concentrations rarely exceeded 8 mM and then, only at 1 wk of age.

In contrast to plasma glucose, plasma insulin increased by 38% from 1 to 5 wk of age (age effect,  $P = .09$ ; Figure 3.1), although the magnitude of the plasma insulin response to feed was not affected ( $P = .19$ ; Table 3.2) by the age of the calf. The gradual divergence of plasma glucose and insulin concentrations with age (Figure 3.1) could have at least two

Table 3.2. Least squares means for plasma glucose and insulin concentrations at weekly intervals prior to (Pre) and 2 h after feeding (Post)<sup>1</sup> in calves supplemented with chromium picolinate.

Treatment <sup>2</sup>	Age, weeks	Glucose			Insulin		
		Pre	Post	change <sup>3</sup>	Pre <sup>4</sup>	Post <sup>4</sup>	change <sup>5</sup>
		—— (mM) ——		(%)	—— (ng/ml) ——		(%)
Control							
	1	5.31 (5.1, 5.5) <sup>6</sup>	6.43 (6.1, 6.8)	21.3 (13, 30)	.28 (.21, .37)	1.97 (1.3, 2.9)	554.8 (297, 955)
	2	5.21 (5.0, 5.4)	6.51 (6.1, 6.9)	24.2 (16, 34)	.30 (.23, .40)	1.01 (.7, 1.5)	221.0 (87, 435)
	3	4.90 (4.7, 5.1)	6.25 (5.9, 6.6)	26.0 (17, 36)	.34 (.25, .44)	1.41 (1.0, 2.1)	177.1 (60, 366)
	4	4.62 (4.4, 4.9)	5.94 (5.6, 6.3)	26.7 (18, 37)	.33 (.25, .44)	1.58 (1.1, 2.3)	374.0 (182, 675)
	5	4.55 (4.3, 4.8)	5.58 (5.2, 5.9)	19.6 (12, 28)	.36 (.27, .47)	1.84 (1.3, 2.7)	378.3 (185, 682)
Chromium							
	1	5.37 (5.1, 5.6)	6.04 (5.7, 6.4)	8.4 (2, 16)	.30 (.22, .40)	1.41 (.9, 2.1)	326.2 (148, 613)
	2	4.99 (4.7, 5.2)	6.26 (5.9, 6.6)	25.1 (16, 35)	.26 (.20, .35)	.93 (.6, 1.4)	218.8 (82, 442)
	3	4.70 (4.4, 4.9)	6.27 (5.9, 6.8)	34.0 (24, 46)	.31 (.23, .42)	1.28 (.8, 2.0)	296.3 (124, 580)
	4	4.48 (4.2, 4.7)	6.06 (5.7, 6.5)	33.1 (23, 45)	.37 (.28, .50)	1.34 (.9, 2.0)	223.2 (81, 460)
	5	4.57 (4.3, 4.8)	5.73 (5.3, 6.1)	20.9 (12, 31)	.45 (.33, .61)	2.40 (1.6, 3.7)	432.7 (206, 800)
P > F <sup>7</sup>							
Treatment		.44	.72	.98	.84	.74	.72
Age		.0001	.002	.003	.09	.004	.19
Treatment x age		.69	.55	.12	.70	.67	.53

(Table con'd.)

<sup>1</sup>For prior to feeding (Pre) variables, n = 21 and 20 calves for control and chromium means, respectively, and for 2 h after feeding (Post) and percentage change variables, n = 17 and 16 calves for control and chromium means, respectively

<sup>2</sup>There were no interactions of treatment or age with sex ( $P > .10$ ).

<sup>3</sup>For statistical analysis, the percentage change in glucose original values were transformed by raising the original values to the .4 power.

<sup>4</sup>For statistical analysis, the Pre and Post insulin original values were transformed by taking the natural log of the original values.

<sup>5</sup>For statistical analysis, the percentage change in insulin original values were transformed by raising the original values to the .1 power.

<sup>6</sup>With the exception of Pre and Post glucose variables, all means are based on analysis of transformed data; consequently, the lower and upper 95% confidence limits for each mean are given in parentheses.

<sup>7</sup>Probability of a greater F-value.

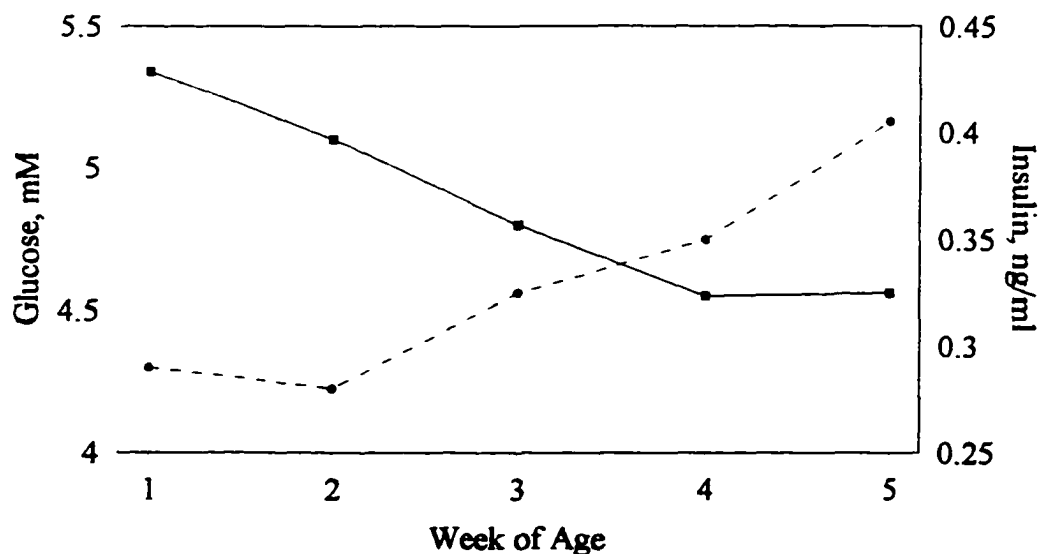


Figure 3.1. Weekly prefeeding concentrations of plasma glucose and insulin. Solid line = glucose; dash line = insulin.

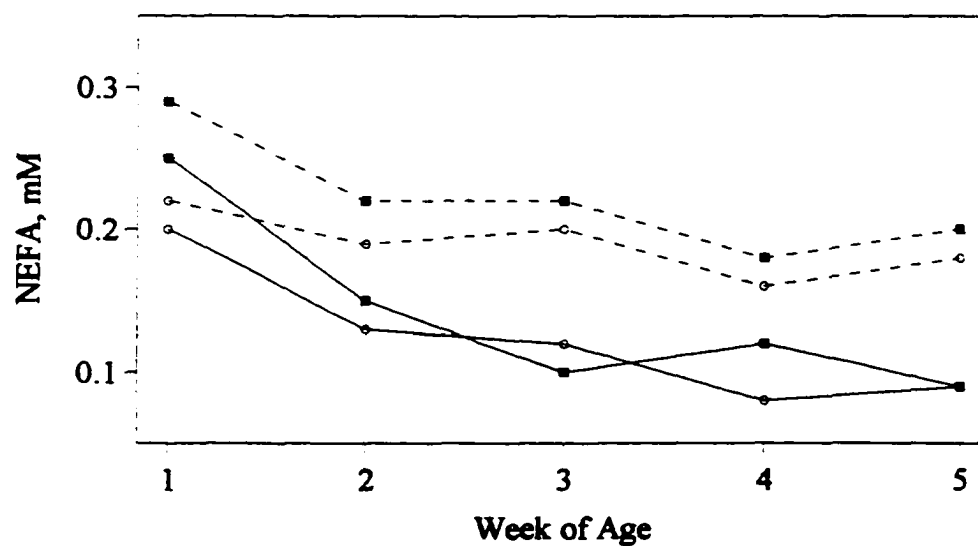


Figure 3.2. Weekly prefeeding and 2 hours after feeding concentrations of plasma nonesterified fatty acids (NEFA). Solid line = basal; dash line = 2 hours after feeding; square = control; circle = chromium picolinate.

unique developmental components. First, insulin insufficiency appears to be a common characteristic of neonates, as pancreatic insulin release is limited for the 1st few d or wk of life. For example the capacity to clear a load of glucose increases measurably within the 1st wk of life in humans (Baird and Farquhar, 1962; Bowie et al., 1963; Falorni et al., 1974), between the 2nd and 4th wk of life in rats (Issad et al., 1987), and between the 7th and 8th wk of life in dogs (Varma et al., 1973). Similarly, Grutter and Blum (1991) reported the mechanisms of insulin secretion may not be fully developed in young calves. Secondly, there is evidence suggesting that tissue sensitivity to insulin is adequate in the young preruminant animal but begins to decline as the rumen begins to develop (Weekes et al., 1983; Metcalf and Weekes, 1990; Grutter and Blum, 1991; Sano et al., 1991). Thus, the data from the present experiment may indicate that, as the calves become older, more insulin was required to maintain a declining homeostatic level of glucose. That is, calves may have gradually become less insulin sensitive with age.

By the time the second IVGTT was conducted at 8 wk of age, prefeeding plasma glucose concentrations were some 20% lower than during the first IVGTT conducted at 2 wk of age (Table 3.3). It was interesting that glucose clearance rates were almost 40% faster at 2 wk of age compared with 8 wk of age when the calves were fully functional ruminants. Although not statistically testable, this may again indicate that the calves were becoming less insulin sensitive. At 2 wk of age, heifers had higher ( $P = .10$ ) glucose clearance rates than bulls; however, this effect had all but disappeared ( $P = .67$ ) by 8 wk of age. Supplemental Cr had no influence on ( $P > .10$ ) on basal plasma glucose, glucose clearance rate or AUC at either 2 or 8 wk of age. When Cr was supplemented as Cr-

Table 3.3. Least squares means for kinetic response variables during i.v. infusions of glucose and propionate in calves supplemented with chromium picolinate<sup>1</sup>.

Item	Treatment		SEM	P > F <sup>2</sup>	Sex		SEM	P > F <sup>2</sup>
	Control	Chromium			Bulls	Heifers		
n	10	11			10	11		
<b>IVGTT<sup>3</sup></b>								
2 weeks of age								
Basal glucose concentrations, mM	5.05	4.90	.22	.64	4.76	5.18	.22	.19
Glucose clearance rate, %/min	2.24	2.22	.38	.96	1.76	2.70	.38	.10
Glucose AUC, min·mM	145	161	29	.71	173	134	29	.36
8 weeks of age								
Basal glucose concentrations, mM	4.02	3.91	.22	.73	3.80	4.13	.22	.31
Glucose clearance rate, %/min	1.59	1.16	.19	.12	1.32	1.43	.19	.67
Glucose AUC, min·mM	301	320	23	.57	314	308	23	.84
<b>PLT<sup>4</sup></b>								
8 weeks of age								
Basal propionate concentrations, mM	.57	.35	.10	.15	.42	.50	.10	.61
Propionate clearance rate, %/min	7.02	8.24	.85	.33	7.75	7.51	.85	.84
Basal glucose concentrations, mM	3.92	3.54	.26	.30	3.64	3.82	.26	.63
Glucose AUC, min·mM	90	70	20	.51	20	139	21	.001

(Table con'd.)

<sup>1</sup>There were no ( $P > .10$ ) treatment interactions.

<sup>2</sup>Probability of a greater F-value.

<sup>3</sup>Kinetic criteria from i.v. glucose tolerance test (IVGTT): basal concentrations immediately prior to challenges; clearance rate computed from 15 to 30 min after glucose challenge and area under the response curve (AUC) from 0 to 60 min.

<sup>4</sup>Kinetic criteria from propionate loading test (PLT): basal concentrations immediately prior to challenge; propionate clearance rate calculated from 5 to 20 min after propionate infusion and glucose area under the response curve (AUC) from 0 to 60 min.



nicotinate or  $\text{CrCl}_3$ , glucose clearance rate during an IVGTT was not affected in 9-wk old Holstein bull calves fed only milk (Kegley et al., 1997a) or in feeder calves (Kegley and Spears, 1995). In contrast, glucose clearance rate during an IVGTT was 40 and 47% faster in steers and heifer feeder calves respectively, when the calves were supplemented with Cr-picolinate (Bunting et al., 1994).

During the PLT, basal levels of propionate and glucose in the blood were similar ( $P > .10$ ) for all calves (Table 3.3). Supplemental Cr affected ( $P > .10$ ) neither the propionate clearance rate nor the blood glucose response (glucose AUC) to the PLT. Although the heifers and bulls cleared their propionate loads at similar rates ( $P = .33$ ), heifers had a much greater ( $P = .001$ ) plasma glucose response to the propionate challenge than did bull calves. When combined with the finding of greater glucose clearance rates during IVGTT by heifers at 2 wk of age, this finding may indicate that there are innate differences in glucose metabolism between males and females, at least within the Holstein breed.

Plasma NEFA concentrations, measured both prior to and 2-h after feeding, declined with age in all calves ( $P = .0001$ ; Table 3.4). However, the magnitude of the increase in blood NEFA concentrations following milk replacer consumption became much greater as the calves grew older (age effect;  $P = .002$ ; Table 3.4). It is worth noting that plasma NEFA concentrations typically decrease in most animals following consumption of meal. This is largely because of insulin's actions to decrease lipolysis and increase reincorporation of fatty acid into adipocytes. Chilliard (1993) noted that NEFA concentrations in ruminants receiving a high level of fat averaged 13% greater than the mean of control groups. Chilliard postulated that circulating NEFA concentrations become elevated because of

Table 3.4. Least squares means for plasma nonesterified fatty acids (NEFA), cortisol, and insulin-like growth factor-1 (IGF-1) concentrations at weekly intervals prior to (Pre) and 2 h after feeding (Post)<sup>1</sup> in calves supplemented with chromium picolinate.

		NEFA			Cortisol	IGF-1
Treatment <sup>2</sup>	Age, weeks	Pre <sup>3</sup>	Post <sup>3</sup>	change <sup>3</sup>	Pre <sup>3</sup>	Pre <sup>3</sup>
		— (mM) —		(%)	— (ng/ml) —	
Control						
	1	.25 (.20, .30) <sup>4</sup>	.29 (.25, .33)	27.8 (1.9, 60.4)	5.92 (4.45, 7.87)	8.87 (7.8, 10.0)
	2	.15 (.12, .18)	.22 (.19, .25)	58.4 (27.3, 97.1)	2.37 (1.78, 3.15)	9.13 (8.0, 10.4)
	3	.10 (.08, .12)	.22 (.19, .25)	99.7 (59.2, 150.5)	.92 (.68, 1.23)	9.38 (8.2, 10.7)
	4	.12 (.10, .15)	.18 (.16, .21)	59.8 (28.4, 98.9)	.70 (.53, .93)	10.99 (9.6, 12.6)
	5	.09 (.07, .11)	.20 (.17, .22)	128.1 (80.1, 188.9)	.58 (.44, .78)	15.23 (13.3, 17.4)
Chromium						
	1	.20 (.16, .24)	.22 (.19, .25)	23.4 (-1.6, 54.9)	7.00 (5.23, 9.38)	7.90 (6.9, 9.1)
	2	.13 (.11, .16)	.19 (.16, .22)	69.4 (31.1, 118.9)	2.43 (1.81, 3.25)	8.91 (7.7, 10.3)
	3	.12 (.10, .15)	.20 (.17, .22)	44.0 (13.7, 82.4)	1.12 (.83, 1.51)	10.34 (9.0, 11.9)
	4	.08 (.07, .10)	.16 (.14, .18)	92.5 (52.1, 143.8)	.89 (.66, 1.21)	12.34 (10.7, 14.2)
	5	.09 (.07, .11)	.18 (.16, .21)	117.5 (69.6, 178.8)	.69 (.51, .93)	16.45 (14.3, 18.9)
P > F <sup>5</sup>						
Treatment		.12	.01	.72	.40	.80
Age		.0001	.0001	.0002	.0001	.0001
Treatment x age		.04	.62	.25	.96	.42

(Table con'd)

<sup>1</sup>For prior to feeding (Pre) variables, n = 21 and 20 calves for control and chromium means, respectively, and for 2 h after feeding (Post) and percentage change variables, n = 17 and 16 calves for control and chromium means, respectively.

<sup>2</sup>There were no interactions of treatment or age with sex ( $P > .10$ ) except for basal NEFA concentrations (treatment x age x sex;  $P = .05$ ).

<sup>3</sup>For statistical analysis, the original values for all variables were transformed by taking the natural log of the original values.

<sup>4</sup>All means are based on analysis of transformed data; consequently, the lower and upper 95% confidence limits for each mean are given in parentheses.

<sup>5</sup>Probability of a greater F-value.

incomplete removal of free fatty acids into tissue adipocytes following the action of lipoprotein lipase on chylomicrons and lipoproteins. The milk replacer used in this study contained 15% fat (DM basis) which would be some five times greater than the level of fat typical of a post-weaning ruminant diet. Compared with control calves, prefeeding plasma NEFA were lower in calves given Cr-supplemented milk replacer in the first wk of life; however, because blood NEFA declined more rapidly in control calves, calves in the two treatments had similar prefeeding plasma NEFA concentrations by 5 wk of age (treatment  $\times$  age;  $P = .04$ ; Figure 3.2). When measured 2-h after feeding, plasma NEFA concentrations from 1 to 5 wk of age were lower for calves fed Cr-supplemented compared with the control milk replacer (.19 vs. .22 mM;  $P = .01$ ; Table 3.4). Supplemental Cr has reduced blood lipid concentrations in humans and laboratory animals (Doisy et al., 1976; Liu and Morris, 1978; Anderson et al., 1988; Mertz, 1992; Mertz, 1993), as well as in ruminants (Samsell and Spears, 1989; Bunting et al., 1994; Kitchalong et al., 1995; Yang et al., 1996). The mechanism of action of Cr on lipid metabolism is not well understood. However, Mertz (1993) suggested that the primary hypolipidemic effects of organic Cr may be simply attributable to the aforementioned effect of insulin in decreasing lipolysis and increasing reincorporation of fatty acids into adipocytes.

Concentrations of plasma cortisol were not affected ( $P > .10$ ) by Cr supplementation or sex of the calves; however, by 5 wk of age, plasma cortisol concentrations were about 10% of the concentrations observed at 1 wk of age (age effect;  $P = .0001$ ; Table 3.4). This is consistent with what has previously been reported for calves (Massip et al., 1977) and human infants (Wittekind et al., 1993), where concentrations have been observed to reach

baseline adult concentrations by 2 to 3 wk of age. Cortisol levels could have an important role in the stress-susceptible neonate because, somewhere between the point of entry of glucose into the cells and its final degradation, cortisol directly delays the rate of glucose utilization (Guyton, 1991). Hence, it is not unrealistic that Cr, via improved binding of insulin to its receptors, might help normalize blood glucose and (or) NEFA concentrations in the presence of high cortisol concentrations.

In chronically stressed cattle, Bennet et al. (1989) reported that high cortisol levels lead to hyperglycemia and elevated free fatty acids. In the short-term increased cortisol can provide a positive benefit by suppressing inflammatory responses; however, over prolonged periods, immune responses can be impaired such that relatively innocuous infections can escalate to the point of extreme morbidity (Goodman, 1988). Moonsie-Shageer and Mowat (1993) first reported that addition of Cr to the feed of “market-transit” stressed feeder calves lowered circulating cortisol concentrations, reduced morbidity, and increased growth during the first month after being transported from market to the experimental site; however, similarly conducted studies have not been able to confirm this response (Mowat et al., 1993; Wright et al., 1994; Chang et al., 1995). The finding that Cr did not affect cortisol concentrations in the present experiment is in agreement with findings that have been reported in sheep (Kitchalong et al., 1995; Forbes et al., 1998) and calves (Bunting et al., 1994; Kegley and Spears, 1995).

Concentrations of plasma IGF-1 were not affected ( $P > .10$ ) by Cr supplementation or sex of the calves; however, by 5 wk of age, plasma IGF-1 concentrations had almost doubled in the calves (age effect;  $P = .0001$ ; Table 3.4). In dairy heifers fed whole milk

through weaning (8 wk of age) and given access to hay and starter beginning at 3 and 4 wk of age, respectively, IGF-1 concentrations were observed to gradually increase from wk 4 to 12 and after wk 12 decreased (Hugi and Blum et al., 1997). To our knowledge, this is the first report of IGF-1 concentrations in growing calves receiving supplemental Cr. Addition of Cr to the rations of lactating dairy cows did not affect IGF-1 concentrations (Burton et al., 1995); however, in yearling ewes, there was a trend for increased IGF-1 concentrations in lambs fed Cr-picolinate (Forbes et al., 1998). Growth hormone concentrations were not affected by Cr supplementation in feeder calves (Bunting et al., 1994). The effects of growth hormone on nutrient metabolism and tissue accretion are largely mediated through IGF-1. Among the specific effects of growth hormone are an increased rate of protein synthesis and increased mobilization of fatty acids from adipose tissue (Guyton, 1991; Granner, 1996). Concentrations of IGF-1 in the blood of farm animals are positively correlated with developments of skeletal mass and with the composition and rate of body tissue deposition (Olsen et al., 1981; Buonomo et al., 1987; Anderson et al., 1988). Changes in circulating concentrations of plasma IGF-1 are primarily regulated by energy balance and secondarily by protein in most animals (Underwood et al., 1986; Ronge et al, 1988; Ronge and Blum, 1989).

## CHAPTER IV

### PERFORMANCE AND METABOLIC RESPONSES OF PRE-WEANED HOLSTEIN CALVES FED A BASAL OR A HIGH LEVEL OF FAT IN MILK REPLACERS SUPPLEMENTED WITH CHROMIUM NICOTINATE

#### Introduction

Chromium (Cr) is well established as an essential trace element for man and laboratory animals (Anderson, 1987; Mertz, 1993). Chromium appears to be an integral component of a molecular complex known as the glucose tolerance factor which facilitates the cellular binding and action of insulin; however, the exact mechanism(s) at the receptor/cellular level has not been clearly defined (Mertz et al., 1974; Govindaraju et al., 1989; Mertz, 1993). There is increasing evidence that Cr may be a required nutrient in livestock diets. Supplemental Cr has improved glucose tolerance and a number of production parameters in swine (Page et al., 1993; Amoikon et al., 1995). In ruminants, positive production responses to supplemental Cr seem to depend upon the presence of stressors, such as, "market-transit" stress (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Mowat et al., 1993) or early lactation (Subiyatno et al., 1996). Supplemental Cr has been reported to improve immune responses and (or) disease resistance in cattle (Burton et al., 1993; Kegley et al., 1996; Kegley et al., 1997b). Supplemental Cr has had only modest effects on glucose tolerance or other indices of carbohydrate metabolism in functional ruminants (Bunting et al., 1994; Kegley and Spears, 1995; Kitchalong et al., 1995; Subiyatno et al., 1996).

Because glucose is absorbed in comparatively large quantities in the neonatal ruminant, the importance of insulin to glucose homeostasis is analogous to that of the

nonruminant. Grutter and Blum (1991) reported that insulin secretory mechanisms may not be fully developed in young calves. In addition, there is evidence that milk-fed calves become insulin resistant with age, resulting in exaggerated postprandial elevations in insulin and glucose in the blood (Hostettler-Allen et al., 1993, 1994). Under these conditions, there is the potential for urinary spillage of blood glucose and reduced efficiency. Therefore, it seems plausible that neonatal calves would be more metabolically responsive to Cr than adult cattle. The limited data that are available with pre-weaned calves are not conclusive regarding the effects of supplemental Cr on carbohydrate metabolism (DePew et al., 1995; Kegley et al., 1997a).

Supplemental fat has been reported to increase insulin resistance in lactating cattle (Palmquist and Moser, 1981). In addition, high-fat milk replacers have been reported to elevate blood glucose concentrations in Holstein calves (Wijayasinghe et al., 1984). In our previous study with pre-weaned calves fed a conventional milk replacer (Chapter 3), supplemental Cr elicited reductions in circulating NEFA, but no apparent effect on glucose homeostasis (i.e., glucose tolerance/insulin sensitivity). In the present study, pre-weaned calves were fed milk replacers with either a conventional or an elevated level of fat. Our objectives were to determine: 1) whether supplemental Cr would have a measurable effect on insulin sensitivity, if greater insulin resistance is induced with added fat; 2) whether supplemental Cr would continue to reduce blood lipid levels when a higher level of dietary fat is eaten.



## Materials and Methods

Animals and Dietary Treatments. Thirty-four Holstein calves (21 bulls and 13 heifers;  $38.5 \pm 6$  kg initial BW), born at the campus dairy farm in Baton Rouge, were used in an experiment approved by the Institutional Animal Care and Use Committee of the LSU Agricultural Center. Although the calves were born over a four-month period (November to February, 1996), the experiment period for each calf was from 4 d of age to around 5 wk of age. Calves were removed from their dams within 24-h of birth, weighed, and individually housed in 2.5-m<sup>2</sup> calf hutches with a 2.3-m<sup>2</sup> wire enclosure attached to the hutch. For the initial 3 d of life, calves received colostrum only (1.9 liters twice daily) and were trained to drink from buckets. Any colostrum not voluntarily consumed was fed by bottle or esophageal feeding tube. Calves readily drinking from buckets and showing no apparent health problems at 3 d of age were divided within birth date and sex peer groups and then randomly assigned to one of four dietary treatment groups.

Dietary treatments, arranged in a 2 x 2 factorial, consisted of milk replacer with either a basal level of fat (15% of DM as fat; BF) or a high level of fat (22% of DM as fat; HF) fed either without (control) or with supplemental chromium (Cr). A commercial milk replacer with a guaranteed analysis of 22% CP and 15% fat (Nurstrate®, Moorman's, Inc., Quincy, IL ) was used for the BF replacer, whereas the HF replacer was formulated using 85% of Nurstrate® and 15% Super calf-kit® (Merrick's, Inc., Middleton, WI), a commercial high-energy milk replacer supplement with a guaranteed analysis of 7%CP and 60% fat. The estimated daily nutrient consumption (based on guaranteed analysis) from the BF and HF replacers for a 36.3 kg calf at birth are presented in Table 4.1. Both replacers

Table 4.1. Estimated daily nutrient consumption<sup>1</sup> from the basal<sup>2</sup> and high-fat<sup>3</sup> milk replacers for a 36.3 kg calf at birth.

Estimated	Milk Replacers	
	Basal Fat	High Fat
Crude Protein, g/d	114.4	114.6 (97.4, 17.2) <sup>4</sup>
Fat, g/d	78.0	126.2 (107.3, 18.9)
Carbohydrate, g/d	327.6	339.2 (288.3, 50.9)
Total, g/d	520	580 (493, 87)

<sup>1</sup>Based on guaranteed analysis.

<sup>2</sup>Nurstrate®, Moorman's, Inc, Quincy, IL.

<sup>3</sup>85% Nurstrate plus 15% Super calf-kit®, Merrick's, Inc, Middleton, WI.

<sup>4</sup>In parentheses are grams provided by Nurstrate and Super Calf-kit, respectively.

were reconstituted to 15% DM using  $40 \pm 1^\circ\text{C}$  tap water and calves receiving the BF and HF replacers received 1.4% and 1.6%, respectively, of replacer DM per kg of BW. The HF replacer was formulated such that calves fed the HF replacer consumed about 60% more fat but similar amounts of carbohydrate and protein as calves fed the BF replacer (Table 4.1). The milk replacer was given in two equal feedings at 0800 and 1600 daily throughout the experiment. Chromium was provided as Cr nicotinate (13.7% Cr; LONZA, Inc., Fairlawn, NJ). On the 1st 2 d of the experiment (d 4 and 5 of age), Cr was included at 5 ppm (DM basis) as a priming dose and for the remainder of the experiment Cr was included at 1 ppm (DM basis).

On the morning following collection of the wk 2 blood samples, calves were offered a commercial calf starter with a guaranteed analysis of at least 16% CP and 2% fat (Purina® commercial calf starter/grower (coarse) BVT 60; Purina Mills, Inc, St. Louis, MO). Starter intake was recorded and fresh feed offered at each feeding. When a calf consumed all of its starter between feedings, an additional 100 g of starter was offered. Body weight (BW) measurements were also obtained at noon when calves were around 2 and 5 wk of age.

Blood Sample Collection and Metabolic Challenges. At 1, 2, 3, 4 and 5 wk of age, blood was collected immediately before (prefeeding) the 1600 feeding by jugular venipuncture into 7-mL evacuated tubes containing potassium oxalate and sodium fluoride (Sherwood Medical, St. Louis, MO). Starter was not removed prior to collection of prefeeding blood samples collected at 3, 4, and 5 wk of age. When calves were around 2 and 5 wk of age, blood samples were also collected by venipuncture prior to and .5, 1, 2,

3, and 4 h after the 1600 replacer feeding. Starter was removed at 1200 when blood samples were collected relative to the 1600 replacer feeding, when calves were 5 wk of age.

Around 5 wk of age, an IVGTT was conducted to measure the calves abilities to clear a glucose load and an epinephrine challenge was conducted to study the relative magnitude of NEFA release and subsequent reincorporation into adipose tissue. The IVGTT was performed after an overnight fast (16 h). Average starter intake for the 3 d preceding and on the d immediately preceding the IVGTT were .71 and .42 kg, respectively. Calves were given their full daily allotment of milk replacer at the evening feeding following the challenges. At 0700, calves were fitted with 14-G jugular catheters (Quik-Cath®, Baxter Healthcare, Deerfield, IL) and were allowed at least 1 h of rest prior to the IVGTT. The IVGTT consisted of infusing a glucose solution (500 mg/kg of BW; 50% w/v solution in sterile saline) as a bolus dose over a 30- to 60-s interval. Prior to infusion of glucose, one blood sample was drawn (0 min) and then glucose was administered (1 mL/kg BW) i.v. through the catheter. Blood was then drawn at 10, 20, 30, 40, 50, 60, 90 and 120 min relative to the glucose infusion.

During the 2-h period between completion of the IVGTT and the epinephrine challenge, the calves were only allowed access to water. The epinephrine challenge consisted of a bolus i.v. infusion of epinephrine (2  $\mu$ g Epinephrine-HCl/kg BW). Two pre-infusion blood samples were drawn (- 20 and 0 min) and then epinephrine administered (.02 mL/kg BW) i.v. through the catheter. Blood samples were then drawn at 10, 20, 40, 60, 80, 100 and 120 min relative to propionate infusion. To ensure that none of the infusion solutions remained in the catheter sheath, following infusion of glucose or epinephrine-HCl,

sterile saline (5 mL) was flushed through the catheter. Catheters were maintained with 6% sodium citrate in sterile saline.

**Blood Analyses.** Plasma was analyzed for glucose (Sigma Tech. Bull. #315, 1989), cholesterol (Sigma Tech. Bull. # 352, 1992), and triacylglycerol (Sigma Tech. Bull # 334, 1995) spectrophotometrically using commercial kits (Sigma Chemical, St. Louis, Mo.). Plasma NEFA concentrations were determined using a commercial enzymatic procedure (NEFA-C Kit, ACS-ACOD Method; Wako Chemicals, USA, Richmond, VA). Plasma insulin was determined by RIA as described by Sticker et al. (1995) and plasma cortisol was determined by a commercial kit (Cortisol RIA Kit No. DSL-2000; Diagnostic Systems Laboratories Inc., Webster, TX.). Samples were assayed in duplicate and measurements resulting in errors greater than 5% were re-analyzed. The exception was that the measurement of blood triacylglycerol and NEFA from the epinephrine challenge were not replicated.

**Statistical Methods and Calculations.** Clearance rates and area under the response curve (AUC) for glucose and insulin were computed for each calf following the IVGTT (Kaneko, 1989). To further evaluate carbohydrate metabolism a computer modeling procedure which has been used to evaluate glucose effectiveness ( $S_G$ ) and insulin sensitivity ( $S_i$ ) in humans was also employed (Watanabe et al., 1995; Bergman, 1997). In general,  $S_G$  is a measure of insulin-independent glucose disappearance from the circulation, whereas  $S_i$  is a measure of insulin-dependent glucose clearance. The computer model was originally developed to evaluate  $S_G$  and  $S_i$ , among other variables, based on glucose and insulin concentrations obtained at specific intervals in response to an i.v. infusion of glucose,

followed by an infusion of insulin that is also given at a specific time. We did not employ the full minimal model procedure, nonetheless, the model was able to provide an estimate of  $S_i$  from the glucose and insulin concentrations obtained from the IVGTT. The validity of the  $S_i$  values obtained and degree of fit by the model was evaluated by J. C. Lovejoy (Lovejoy, personal communication).

Data for initial BW, ADG, starter intake, kinetic analysis of metabolite challenges and relative to feeding responses were analyzed by least-squares ANOVA and PROC GLM of SAS (SAS®, 1989). The model was a factorial including Cr level, fat level, and Cr by fat level interaction. Data for prefeeding (weekly) and responses relative to milk feedings (2 and 5 wk of age) for hormone and metabolite concentrations were analyzed as a split-plot. In the whole plot, Cr level, fat level, and Cr by fat level were tested against calf (Cr x fat level) means square. In the sub-plot, the effect of time and all interactions of time with Cr and fat level were tested against the residual mean square. The PROC UNIVARIATE procedure of SAS (SAS® 1989) was used to test for normality and a Shapiro-Wilks test value of 0.1 was selected for rejection of the null hypothesis. When normality was rejected, data transformation was used to achieve normality and transformed values analyzed. Means are reported in original units and all statistical analyses were conducted using the GLM procedures of SAS (SAS® 1989).

## **Results and Discussion**

**Background.** In preweaning, conventionally-fed Holstein calves consuming a commercial 15% fat milk replacer supplemented with chromium picolinate, we observed NEFA concentrations were reduced (DePew et al., 1995). Although none of the indices

used to evaluate whether Cr had influenced glucose tolerance and (or) insulin effectiveness were significant, our interpretation of the results, as a whole, led us to believe that calves receiving supplemental Cr may have maintained insulin sensitivity longer (age). Kegley (1997a) and co-workers observed that supplementation of Cr to the diets of intensively-fed Holstein calves consuming a 20% fat milk replacer had no influence on most of the indices used to evaluate whether Cr affected glucose tolerance and (or) insulin effectiveness. However, based on the glucose response, following an i.v. infusion of insulin, these investigators stated, “tissues from calves that were fed Cr might have been more sensitive to the insulin, or the insulin might have had a longer lasting effect in these calves”. Because our study showed reduced NEFA concentrations, and both of the previously mentioned studies hinted toward insulin sensitivity being maintained longer in Cr-fed calves, we included dietary fat level as a second factor under the premise that calves fed the higher level of fat would have greater circulating blood lipid concentrations and become more insulin insensitive at an earlier age. This would enable us to develop a clearer picture of whether the hypolipidemic effects and improved glucose tolerance/insulin sensitivity effects, that have been attributed to the supplementation of Cr in humans with non-insulin dependent diabetes, (Wallach, 1985; Mertz, 1993) would also cause similar metabolic changes in the conventionally-fed, pre-weaned dairy calf.

**Performance.** Other than minor, controllable bouts of scours, calves were healthy throughout the experiment. The rates of gain achieved by the calves in the current experiment were comparable with and, in most instances, better than other investigators have reported for calves of similar ages and when similar feeding regimes were employed

(Doppenberg and Palmquist, 1991; Quigley et al., 1991; Quigley and Bernard, 1992; Kegley et al., 1997a). No dietary interactions ( $P > .10$ ) were observed among any of the performance criteria; therefore, only treatment effects are reported (Table 4.2). Chromium supplementation did not affect ( $P > .10$ ) ADG, ADG:DMI, or starter intake during any of the feeding periods analyzed; however, while calves were fed milk alone there was a tendency for ADG ( $P = .16$ ) and ADG:DMI ( $P = .16$ ) to be greater in Cr-fed compared to control-fed calves. In pre-weaned Holstein calves, similar responses were observed when Cr-picolinate was supplemented in the diet of conventionally-fed calves (DePew et al., 1995) and when  $\text{CrCl}_3$  or a Cr-nicotinic acid complex was supplemented in the diet of intensively-fed calves (Kegley et al., 1997a). In young (6 to 8 wk of age) weaned calves, no difference in ADG over a 51-d period was observed between control-fed calves and calves fed a high-Cr yeast supplemented starter (Arthington et al., 1997). Studies including supplementation of various Cr compounds to the diets of ruminating calves (Bunting et al., 1994; Wright et al., 1994; Chang et al., 1995; Kegley and Spears, 1995; Mathison and Engstrom, 1995) and lambs (Fornea et al., 1994; Kitchalong et al., 1995; Forbes et al., 1998) have also reported no effect on rate of gain, DMI, or feed efficiency.

In ruminants, the greatest potential for supplemental Cr to increase performance (i.e., growth and milk production) would seem to be in animals in which metabolic demand is high (i.e., transit/market, intensive handling, reintroduction with a new group of animals, early lactation). Supplementation of various Cr compounds to the diets of “market-transit” stressed feeder calves had no effect on growth performance in some studies (Wright et al., 1994; Chang et al., 1995; Kegley and Spears, 1995; Mathison and Engstrom, 1995). In



Table 4.2. Effect of chromium nicotinate (CrNIC) and fat level on body weight gain and starter intake in pre-weaned, conventionally-fed Holstein calves<sup>1</sup>.

Item	Treatment			P-value <sup>2</sup> for treatment	Fat Level			P-value <sup>2</sup> for fat level
	Control	CrNIC	SEM		Basal	High	SEM	
n	17	17			16	18		
Initial BW, kg	37.7	39.3	1.5	.48	38.3	38.6	1.5	.88
ADG, kg								
week 0 to 2 <sup>3</sup>	.18	.23	.03	.16	.17	.23	.03	.10
week 2 to 5 <sup>4</sup>	.48	.50	.04	.63	.48	.50	.04	.60
week 0 to 5	.36	.39	.02	.28	.36	.40	.02	.26
ADG:DMI, kg/d								
week 0 to 2	.31	.40	.04	.16	.32	.38	.04	.32
week 2 to 5	.51	.50	.03	.85	.51	.50	.03	.77
week 0 to 5	.46	.48	.02	.63	.47	.47	.02	.94
Starter intake, kg of DM/d <sup>5</sup>	.38	.42	.03	.37	.39	.40	.03	.85

<sup>1</sup>There were no ( $P > .10$ ) dietary treatment interactions.

<sup>2</sup>Probability of a greater F-value.

<sup>3</sup>Milk-fed only.

<sup>4</sup>Starter plus milk-fed.

<sup>5</sup>Week 2 to 5.

contrast, others reported improvements in some growth performance criteria, although, in such cases, the effects were generally limited to the first few wk after the calves arrived (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Mowat et al., 1993; Mathison and Engstrom, 1995; Kegley et al., 1997b). Why Cr supplementation results in improved growth performance, in some but not other similarly conducted experiments, is a matter of speculation. However, an animal's individual Cr status prior to receiving supplemental Cr, the bioavailability and biological activity of Cr compounds supplemented, and stressor variables, are frequently suggested as contributing factors. An explanation for the "stressor" association appears to stem from studies in man and laboratory animals that showed that as demand and (or) intensity of the demand increases, the body responds by increasing its mobilization of stored Cr and, once mobilized, any Cr that is not used, is excreted (Anderson et al., 1993). The likelihood of observing an effect from Cr supplementation under such a situation may therefore be heightened, because there is greater potential for Cr body stores to become depleted faster than can be replenished by the Cr that is available in the base feedstuffs.

In the current experiment, a colostrometer was used to ensure that only high quality colostrum ( $> 70$  mg of immunoglobulins/mL) was fed, and care was taken to ensure it was fed at recommended levels and within the recommended time (Donovan, 1992; Morrill, 1992). Although immunoglobulin concentrations were not measured in the calves, it appears that adequate immunoglobulin transfer was achieved and adequate nutrients were supplied to the calves, such that health problems were minimized. Speculatively, it is possible that, because only healthy calves were initially included in the current experiment,

and because only minor, controllable bouts of scours were observed throughout the experiment, minimal demands (beyond normal growth requirements) were placed on this group of young calves. As a result, no significant improvements in growth performance were detectable. Whether Cr supplementation would provide greater benefit for calves raised under more commercial dairy farm settings needs to be investigated as Heinrichs (1994) and co-workers reported that 75.5% of dairy calves on US farms receive less than adequate amounts of colostrum within the first 24-h of birth. In addition, this statement assumes that calves were fed an acceptable quality colostrum (minimum of 50 mg of immunoglobulins/mL; Scott and Fellah, 1983) that ensured that adequate levels of immunoglobulins were received by each calf.

Fat level of the replacer did not affect ( $P > .10$ ) ADG, ADG:DMI, or starter intake over the entire 5 wk experimental period or during the period after the calves were given access to starter. However, while calves were fed milk alone, ADG ( $P = .10$ ) was greater in calves fed the HF replacer compared with those fed the LF replacer. Wijayasinghe et al. (1984) reported that intensively-fed calves receiving high (30%) versus low-fat (3%) milk replacers gained weight at similar rates from 1 to 3 wk of age; however, after the 3rd wk, calves receiving the high-fat milk replacer gained weight faster than calves receiving the low-fat milk replacer. Scibilia (1987) and co-workers reported that ADG increased linearly with fat level (10%, 17.5%, 25%) of the of the milk replacer during the first 3 wk of life, although the response was more dramatic when calves were housed at temperatures below compared with within the zone of thermal neutrality.

Weekly Changes in Plasma Metabolites and Hormones. Irrespective of dietary treatments, from 1 to 5 wk of age, plasma concentrations for glucose and NEFA decreased ( $P = .001$ ; Figures 4.1 and 4.3) and plasma concentrations for triacylglycerol and cholesterol increased ( $P = .0001$ ; Figures 4.4 and 4.5). Over this period, plasma insulin concentrations were more stable; although, there was a slight tendency ( $P = .17$ ; Figure 4.2) for an increase. For glucose, NEFA and insulin, these age related changes agree with a similarly conducted experiment from our lab (DePew et al., 1995). In addition, other researchers have also observed similar age related changes for all the aforementioned metabolites and hormones (Shannon and Lascelles, 1966; Doppenberg and Palmquist, 1991; Quigley, et al., 1991; Quigley and Bernard, 1992; Kuehn et al., 1994; Hugi and Blum, 1997).

Except for cholesterol (see below), no three-way interactions ( $P > .10$ ) among Cr, fat level, and age were observed for the blood variables measured (Figures 4.1, 4.2, 4.3, 4.4, 4.5), and no interactions ( $P > .10$ ) between Cr and fat level were observed for the blood variables when averaged across the 5 wk. In addition, few interactions of Cr with age or fat with age were observed; therefore, sampling dates were combined and only the independent effects of Cr and fat level, for the various blood variables, are shown (Table 4.3).

At 1 wk of age, plasma insulin concentrations were similar for calves in all dietary treatment groups, however, by wk 2, and lasting through wk 5, a gradual divergence was observed with plasma concentrations tending to be lower for calves fed Cr-supplemented replacer compared with those fed control replacer (Cr x age trend;  $P = .11$ ; Figure 4.2). In

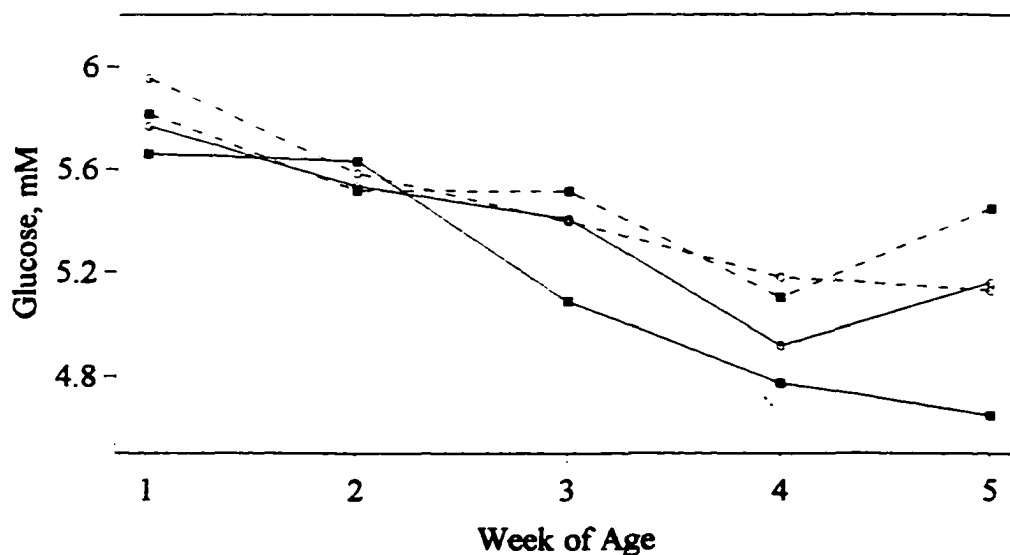


Figure 4.1. Weekly concentrations of plasma glucose. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

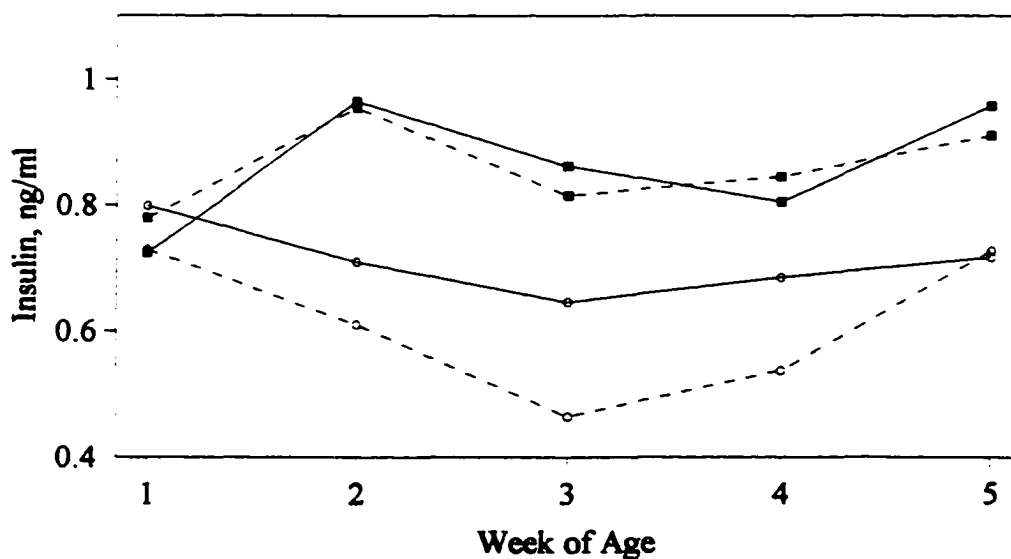


Figure 4.2. Weekly concentrations of plasma insulin. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

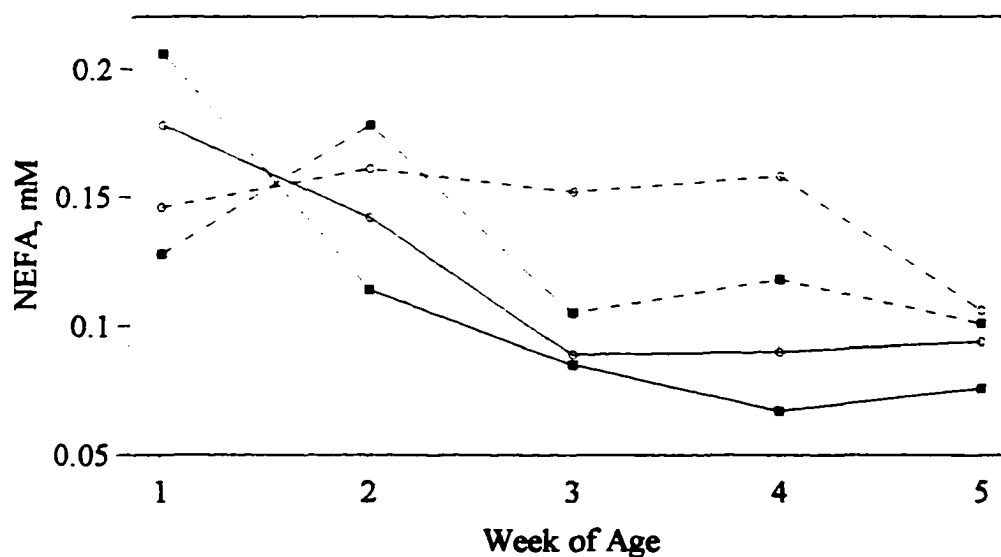


Figure 4.3. Weekly concentrations of plasma nonesterified fatty acids. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

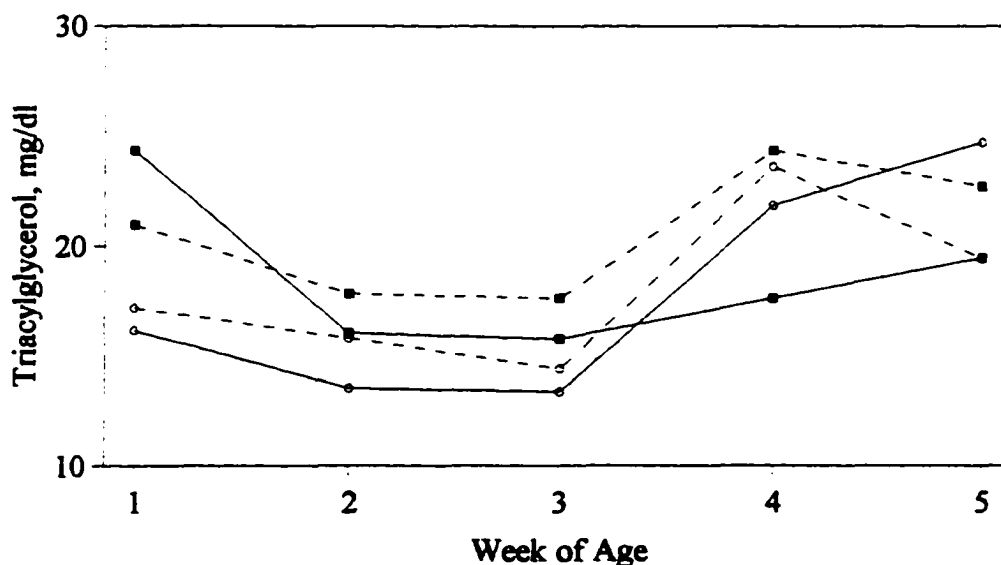


Figure 4.4. Weekly concentrations of plasma triacylglycerol. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

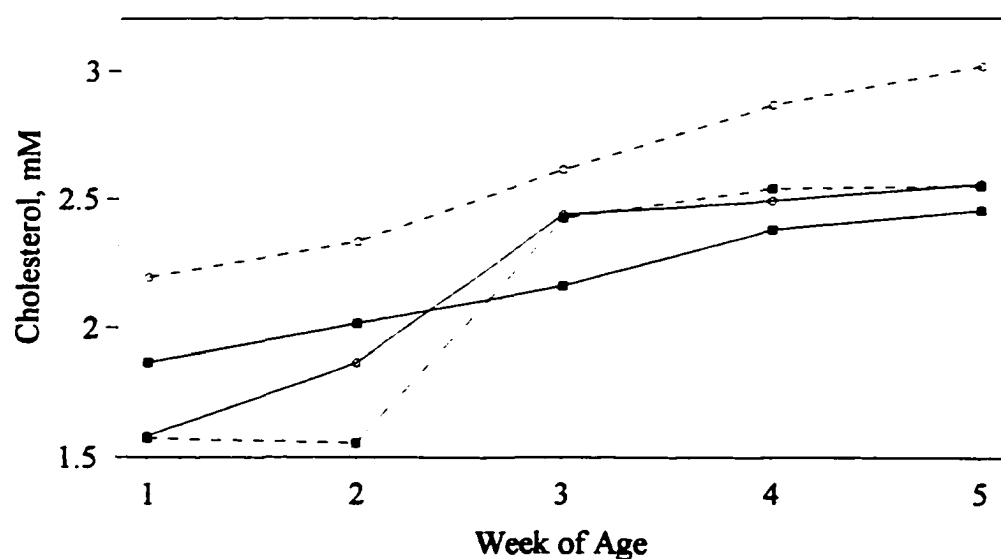


Figure 4.5. Weekly concentrations of plasma cholesterol. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

Table 4.3. Effect of chromium nicotinate (CrNIC) and fat level on prefeeding plasma glucose, insulin, nonesterified fatty acids (NEFA), triacylglycerol (TG), and cholesterol concentrations in pre-weaned, conventionally-fed Holstein calves<sup>1,2,3</sup>.

Item	Treatment		P-value <sup>4</sup> for treatment	Fat Level		P-value <sup>4</sup> for fat level
	Control	CrNIC		Basal	High	
n	17	17		16	18	
Glucose, mM	5.32 (5.15, 5.49) <sup>5</sup>	5.40 (5.24, 5.57)	.47	5.26 (5.09, 5.43)	5.46 (5.30, 5.63)	.09
Insulin, ng/ml <sup>6</sup>	.86 (.59, 1.17)	.66 (.42, .94)	.30	.78 (.52, 1.09)	.73 (.48, 1.02)	.78
NEFA, mM <sup>7</sup>	.11 (.10, .13)	.13 (.11, .14)	.10	.11 (.09, .12)	.13 (.12, .15)	.01
TG, mg/dl <sup>8</sup>	19.6 (15.7, 23.9)	17.8 (14.1, 21.9)	.53	18.1 (14.3, 22.4)	19.3 (15.5, 23.4)	.67
Cholesterol, mM <sup>9</sup>	2.14 (1.92, 2.38)	2.39 (2.15, 2.63)	.14	2.18 (1.94, 2.42)	2.35 (2.13, 2.58)	.28

<sup>1</sup>Mean of samples taken at 1, 2, 3, 4, and 5 weeks of age.

<sup>2</sup>Irrespective of age there were no ( $P > .10$ ) dietary treatment interactions.

<sup>3</sup>There were no interactions with age ( $P > .10$ ) except for plasma NEFA (fat x age;  $P = .0001$ ) and cholesterol (treatment x fat x age;  $P = .01$ ) concentrations.

<sup>4</sup>Probability of a greater F-value.

<sup>5</sup>With the exception of glucose, all means are based on analysis of transformed data; consequently, the lower and upper 95% confidence limits for each mean are given in parentheses.

<sup>6</sup>For statistical analysis, plasma insulin concentrations were transformed by raising the original values to the .7 power.

<sup>7</sup>For statistical analysis, plasma NEFA concentrations were transformed by taking the natural log of the original values.

<sup>8</sup>For statistical analysis, plasma triacylglycerol concentrations were transformed by raising the original values to the .5 power.

<sup>9</sup>For statistical analysis, plasma cholesterol concentrations were transformed by raising the original value to the .7 power.



contrast, plasma glucose concentrations in the Cr-fed and control-fed calves declined at a similar rate from wk to wk ( $P = .99$ ; Figure 4.1). The lack of a Cr treatment difference for plasma glucose coupled with the divergence in plasma insulin between Cr-fed and control-fed calves suggests that insulin sensitivity may have been maintained longer in Cr-fed calves. In mid-gestating sows fed diets supplemented with Cr-picolinate, no differences in plasma glucose concentrations were observed (Lindemann et al., 1995). However, they did observe lower insulin and insulin to glucose ratios in the Cr-fed compared with the control-fed sows and interpreted this as evidence that Cr supplementation improved insulin action. In the present experiment, no Cr or interaction of Cr by age effects ( $P > .10$ ; data not shown) were observed for plasma insulin to glucose ratio; however, when the ratios were averaged across the 5 wk, the ratio was numerically but not significantly smaller (.14 vs. .19;  $P = .17$ ; data not shown) for the Cr-fed calves compared with the control-fed calves. In agreement with our findings, Kegley et al. (1997a) reported that neither supplemental  $\text{CrCl}_3$  nor a Cr-nicotinic acid complex affected prefeeding plasma glucose concentrations in milk-fed Holstein calves when there were 28 or 56 d old. In the aforementioned study, insulin was not reported for d 28 or 56; however, when calves were 9 to 10 wk old, plasma insulin concentrations (6 h after the preceding meal) were lower in Cr-fed compared with control-fed calves; again, plasma glucose concentrations were unaffected. This observation tends to agree with the present experiment, such that, Cr did not induce any change in plasma glucose concentrations, but plasma insulin concentrations (8 to 10 h after the preceding meal) tended to be lower in Cr-fed compared with control-fed calves.

The decline in plasma NEFA concentrations from wk to wk was similar between Cr-fed and control-fed calves (Cr x age;  $P = .51$ ; Figure 4.3). Bunting et al. (1994) also reported supplementing Cr, as Cr picolinate, in the ration of ruminating steers and heifers for a period of 8 wk, did not affect plasma NEFA concentrations (Bunting et al., 1994). In contrast, ruminating lambs, fed diets supplemented with Cr picolinate, had lower plasma NEFA concentrations at 2, 7, and 11 wk (Kitchalong et al., 1995) and 11 and 22 d (Forbes et al., 1998) after supplementation was initiated, compared with control-fed lambs. Studies with ruminant and nonruminant animals alike have typically observed that supplemental Cr tends to either reduce (Samsell and Spears, 1989; Ward et al., 1995; DePew et al., 1995; Kitchalong et al., 1995; Amoikon et al., 1995; Ward et al., 1997; Forbes et al., 1998) or have no effect on plasma NEFA concentrations (Bunting et al., 1994; Ward et al., 1995; Besong et al., 1996; Yang et al., 1996; DePew et al., 1996). Therefore, observing that plasma NEFA concentrations (averaged across the five wk) were greater ( $P = .10$ ; Table 4.3) in Cr-fed compared with control-fed calves was not expected.

Over the weekly sampling dates, plasma cholesterol concentrations in calves fed Cr-supplemented replacer did not differ ( $P = .93$ ) from those of calves fed control replacer; however, there was a Cr by fat by age effect ( $P = .01$ ; Figure 4.5). This latter effect seemed more apparent while calves received only milk. In essence, calves fed the Cr-supplemented, HF replacer had higher plasma cholesterol concentrations compared with calves fed the control, HF replacer; whereas, calves fed the Cr-supplemented, BF replacer had lower plasma cholesterol concentrations compared with calves fed the control, BF replacer. The cholesterol-lowering ability of organic Cr complexes has been shown in both monogastrics

(Mertz, 1993; Page et al., 1993), and ruminants (Bunting et al., 1994; Kitchalong et al., 1995), but again this response is not always observed (Ward et al., 1997; Forbes et al., 1998). Kegley et al. (1997a) reported that supplementing Cr as either  $\text{CrCl}_3$  or a Cr-nicotinic acid complex in the milk replacer fed to milk-only-fed calves did not affect postfeeding (3 h) serum cholesterol concentrations at any time during the initial 9 to 10 wk of life.

Neither Cr nor Cr by age effects ( $P > .10$ ; Figure 4.4) were observed for plasma triacylglycerol concentrations. Similar observations have been reported in lambs (Kitchalong et al., 1995; Forbes et al., 1998) and pigs (Lindemann et al., 1995). To our knowledge, supplementing Cr to livestock has not been observed to affect plasma triacylglycerol concentrations. However, when Holstein cows were fed Cr-picolinate, beginning 30 d prepartum, it has been reported that liver triacylglycerol levels (d 30 of lactation) were lower in the Cr-fed compared with the control-fed cows (Besong et al., 1996). In men classified as being either ketosis-prone; ketosis-resistant, nonobese; and ketosis-resistant, obese, Rabinowitz et al. (1983) reported no change in serum triacylglycerol concentrations following 4 mo of Cr supplementation as  $\text{CrCl}_3$ , a Brewer's yeast that contained Cr as GTF, or a Brewer's yeast extract without GTF. By contrast, Abraham et al. (1992) reported that supplemental Cr, as  $\text{CrCl}_3$ , for a period of 7 to 16 mo lowered serum triacylglycerol concentrations. In non-insulin-dependent diabetes mellitus patients, Lee and Reasner (1994) also reported that triacylglycerol concentrations were significantly reduced following 2 months of Cr supplementation as Cr picolinate.

No interactions between fat level and age were observed for plasma insulin, triacylglycerol, and cholesterol concentrations ( $P > .10$ ; Table 4.3). No interaction between fat level and age was also observed for plasma glucose concentrations ( $P = .59$ ; Figure 4.1); however, when concentrations were averaged across wk, calves fed HF replacer had greater ( $P = .09$ ; Table 4.3) plasma glucose concentrations compared with calves fed BF replacer. Figure 4.1 shows that the divergence in glucose levels between calves fed HF and LF milk replacers did not begin to occur until 3 wk of age. In humans fed diets considered moderate in dietary fat (30 - 40% of dietary energy from fat), it has been shown that insulin sensitivity begins to decrease and hyperglycemia begins to develop at around the second to third wk after the dietary regimen begins (Fukagawa et al., 1990; Swinburn et al., 1991). Feeding of a diet where 60% of the energy is provided by fat has been shown to reduce insulin sensitivity within days after the dietary regimen is begun (Sidery et al., 1990). In rats, insulin sensitivity has also been shown to increase within wk after reducing dietary fat from 40 to 30% of dietary energy (Harris and Kor, 1992). In intensively-fed calves, Wijayasinghe et al. (1984) reported that plasma glucose concentrations were greater in calves receiving milk replacer containing higher levels of fat. Although the difference in fat levels fed in the Wijayasinghe study were more extreme (3 vs. 30%), they suggested that the greater dietary availability of glucose in the lower-fat, higher-carbohydrate-fed calves may have caused greater release of insulin, and this in turn resulted in maintenance of lower steady state plasma glucose concentrations.

To our knowledge, this is the first study to report on the effects of Cr supplementation in livestock fed diets with added fat. At 1 wk of age, plasma NEFA

concentrations were lower in calves fed HF replacer than in calves fed BF replacer; however, from wk 2 through 5 of age, plasma NEFA concentrations were greater in calves fed HF replacer compared with those fed BF (fat x age;  $P = .0001$ ; Figure 4.3). Jaster et al. (1992) reported no effect of added fat (113 and 226 g) on plasma NEFA concentrations in calves fed only milk during the first 6 wk of life. In humans, increases in blood lipids have commonly been observed to be linearly correlated with dietary fat intakes (Harris and Jones, 1991).

Changes in Plasma Metabolites and Hormones in Response to a Milk Feeding Before Calves had Access to Starter. Changes over time and selected response variables (prefeeding concentrations, peak concentrations, and area under the curve) for plasma glucose, insulin, NEFA, and triacylglycerol from samples taken relative to a milk feeding, before calves had access to starter, are presented in Figures 4.6 through 4.9, and Table 4.4, respectively. No Cr by time effect ( $P = .21$ ; Figure 4.6) was observed for plasma glucose; however, prefeeding and peak postfeeding plasma glucose concentrations were greater ( $P < .01$ ; Table 4.4) in the Cr-fed compared with the control-fed calves. In calves fed only milk, Kegley et al. (1997a) also showed that Cr supplementation (as  $\text{CrCl}_3$  or a Cr-nicotinic acid complex) had no effect on pre- or postfeeding (1 h) plasma glucose concentrations taken at various times during the first nine wk of life. In addition, plasma glucose concentrations relative to a milk feeding, when calves were 9 to 10 wk old, did not differ between Cr-fed compared with control-fed calves. In weaned lambs, Kitchalong et al. (1995) also reported that supplemental Cr, as Cr picolinate, did not affect pre- and postfeeding (3 h) plasma glucose concentrations at wk 2, 7, and 11 after supplementation

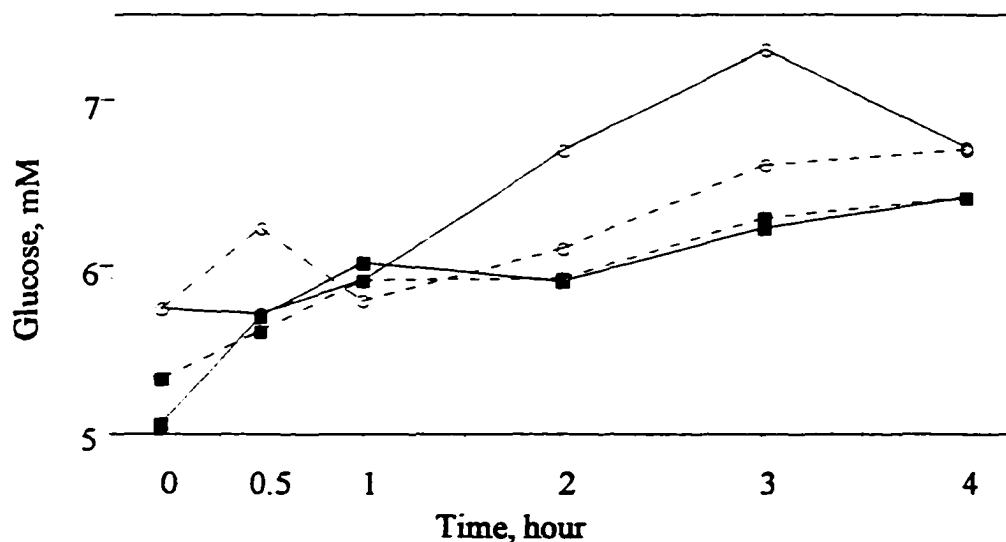


Figure 4.6. Changes in plasma glucose concentrations relative to a milk feeding at 2 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

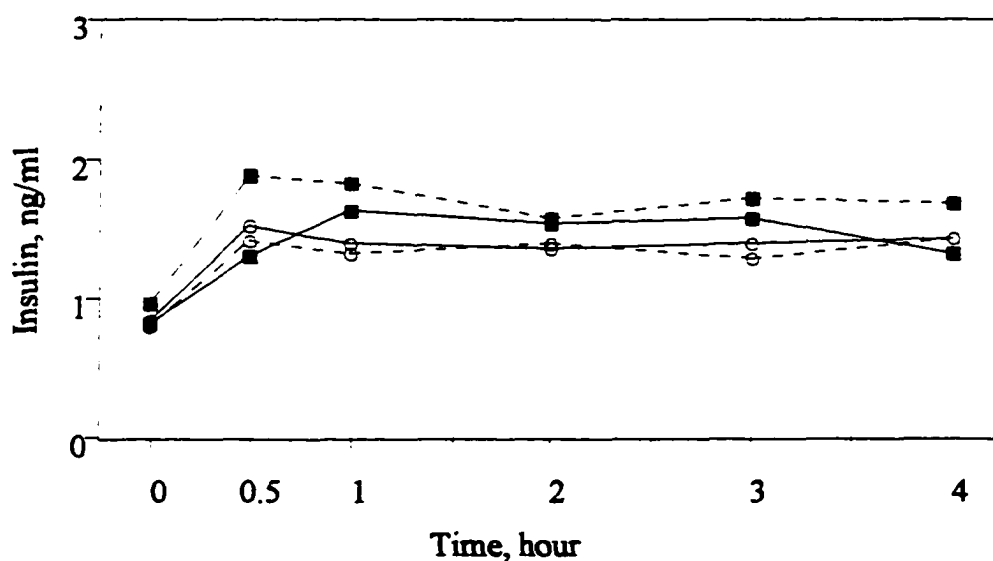


Figure 4.7. Changes in plasma insulin concentrations relative to a milk feeding at 2 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

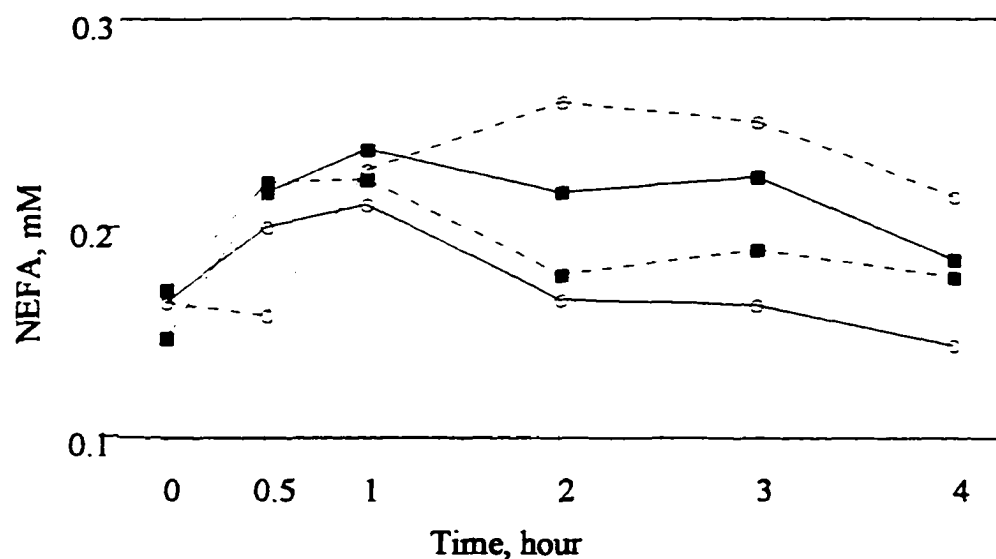


Figure 4.8. Changes in plasma nonesterified fatty acid (NEFA) concentrations relative to a milk feeding at 2 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

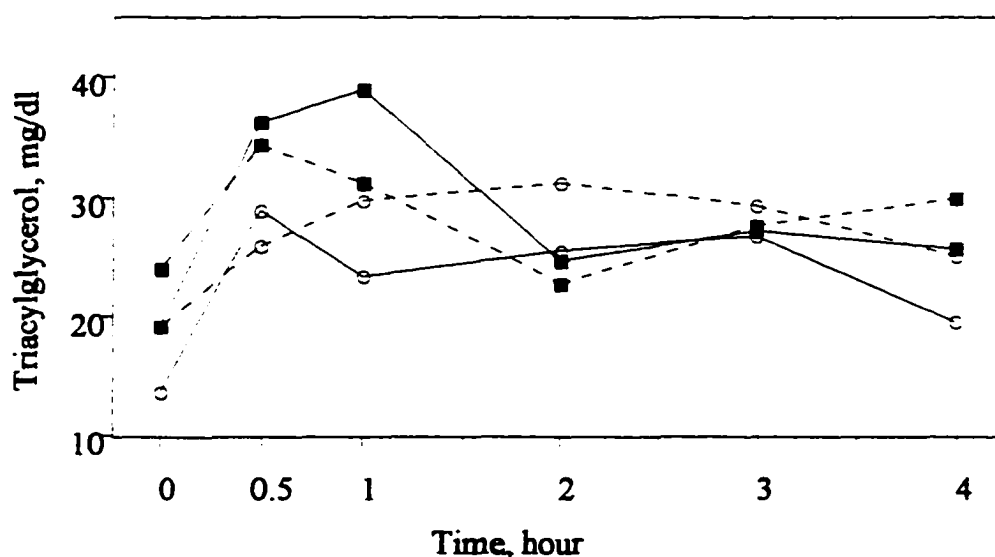


Figure 4.9. Changes in plasma triacylglycerol concentrations relative to a milk feeding at 2 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

Table 4.4. Effect of chromium nicotinate (CrNIC) and fat level on plasma glucose, insulin, nonesterified fatty acids (NEFA) and triacylglycerol responses relative to consumption of milk replacer in 2 week old pre-weaned, conventionally-fed Holstein calves<sup>1</sup>.

Item	Treatment			P-value <sup>2</sup> for Treatment	Fat Level			P-value <sup>2</sup> for Fat Level
	Control	CrNIC	SEM		Basal	High	SEM	
n	17	17			16	18		
<b>Glucose</b>								
Basal concentration, mM <sup>3</sup>	5.2	5.8	.12	.004	5.4	5.5	.12	.46
Peak concentration, mM <sup>4</sup>	6.7	7.5	.20	.01	7.3	6.9	.20	.11
Response curve area, h·mM <sup>5</sup>	3.3	2.6	.54	.40	3.5	2.4	.54	.17
<b>Insulin</b>								
Basal concentration, ng/ml	.98	.94	.15	.88	.92	1.0	.15	.72
Peak concentration, ng/ml	2.4	2.4	.39	.97	2.4	2.4	.39	.99
Response curve area, h·ng/ml	3.0	2.7	.52	.63	2.9	2.8	.52	.86
<b>NEFA</b>								
Basal concentration, mM	.17	.17	.02	.89	.17	.18	.01	.57
Peak concentration, mM	.28	.28	.02	.93	.27	.29	.02	.60
Response curve area, h·mM	.20	.16	.05	.65	.17	.19	.05	.59
<b>Triacylglycerol</b>								
Basal concentration, mM	24.4	19.3	3.4	.30	19.5	24.2	3.4	.34
Peak concentration, mM	45.5	38.3	4.5	.27	40.7	43.1	4.5	.70
Response curve area, h·mM	28.2	38.4	9.0	.43	38.2	28.4	9.0	.45

(Table con'd)



<sup>1</sup>There were no ( $P > .10$ ) dietary treatment interactions, except for NEFA area under the response curve (treatment x fat;  $P = .002$ ).

<sup>2</sup>Probability of a greater F-value.

<sup>3</sup>Basal concentrations = one sample taken immediately prior to offering milk.

<sup>4</sup>Highest point following consumption of milk.

<sup>5</sup>Response curve area = area-under the response curve relative to basal levels computed using trapezoidal geometry for the time period 0 to 4 h after offering milk.

was begun. Prefeeding plasma glucose concentrations did not differ ( $P = .46$ ; Table 4.4) between HF-fed and BF-fed calves and no fat by time interaction ( $P = .53$ ; Figure 4.6) was observed; however, for plasma glucose there was a tendency ( $P = .11$ ; Table 4.4) for peak concentrations to be greater for calves fed BF replacer compared with those fed the HF replacer. In pre-weaned dairy calves fed milk replacer containing either 5% or 25% fat, no differences were observed in plasma glucose concentrations 1 h after the calves had consumed their respective milk replacers (Bazin and Brisson, 1976). During the milk feeding at 2 wk of age, none of the response variables (prefeeding concentration, peak concentration height, and AUC; Table 4.4) and no dietary treatment by time effects ( $P > .10$ ; Figure 4.7) were observed for plasma insulin concentrations. In milk only fed calves, Kegley et al. (1997a) also showed that Cr supplementation, as  $\text{CrCl}_3$  or a Cr-nicotinic acid complex, had no effect on prefeeding plasma insulin concentrations or changes in plasma insulin concentrations when milk replacer was fed. Prefeeding plasma NEFA concentrations were not different ( $P > .10$ ; Table 4.4) between dietary treatments; however, a Cr by fat by time interaction ( $P = .0001$ ; Figure 4.8) was observed in response to the feeding such that, NEFA concentrations increased in CrNIC, HF-fed calves compared with control, HF-fed calves and decreased in CrNIC, BF-fed calves compared with control, BF-fed calves. As observed in Table 4.4, the AUC ( $P = .0002$ ) and peak concentrations ( $P = .17$ ) also showed that the level of dietary fat influenced how plasma NEFA responded in the Cr-fed compared with the control-fed calves. Following the milk feeding at 2 wk of age, none of the response variables (prefeeding concentration, peak concentration height, and AUC; Table 4.4) for plasma triacylglycerol were affected by dietary treatments; however, in response to the milk

feeding, plasma triacylglycerol concentrations did not increase (Cr x time;  $P = .02$ ; Figure 4.9) as much in the Cr-fed as in the control-fed calves.

Changes in Plasma Metabolites and Hormones in Response to a Milk Feeding After Calves had Access to Starter. Changes over time and selected response variables for glucose, insulin, NEFA, and triacylglycerol from samples, taken relative to a milk feeding after calves had access to starter for 3 wk, are presented in Figures 4.10 through 4.13, and Table 4.5. No Cr by time effects ( $P > .10$ ; Figures 4.10, 4.11, 4.12, 4.13) were observed and Cr supplementation alone did not affect ( $P > .10$ ; Table 4.5) prefeeding, peak postfeeding, or AUC for any of the metabolites or hormones. A fat by time interaction ( $P = .68$ ; Figure 4.10) was not observed for plasma glucose; however, prefeeding plasma glucose concentrations tended to be greater ( $P = .13$ ; Table 4.5) and peak postfeeding plasma glucose concentrations were greater ( $P = .02$ ; Table 4.5) in the HF-fed compared to the BF-fed calves. For plasma insulin, a fat by time interaction ( $P = .02$ ; Figure 4.11) was observed such that concentrations increased more in the HF-fed calves during the initial h after feeding compared with the BF-fed calves. When calves were fed the BF milk replacer, prefeeding plasma insulin concentrations were higher in calves receiving supplemental Cr compared with those fed the control; whereas, when calves were fed the HF milk replacer, prefeeding plasma insulin concentrations were lower in calves receiving supplemental Cr compared with those fed the control ( $P = .10$ . Cr x fat trend; Figure 4.11). For plasma NEFA, a Cr by fat by time interaction ( $P = .03$ ; Figure 4.12) was observed such that, in the calves fed the BF replacer, less of a rise in plasma NEFA concentration was observed in the Cr-fed compared with the control-fed calves; whereas, in the calves fed the HF replacer,

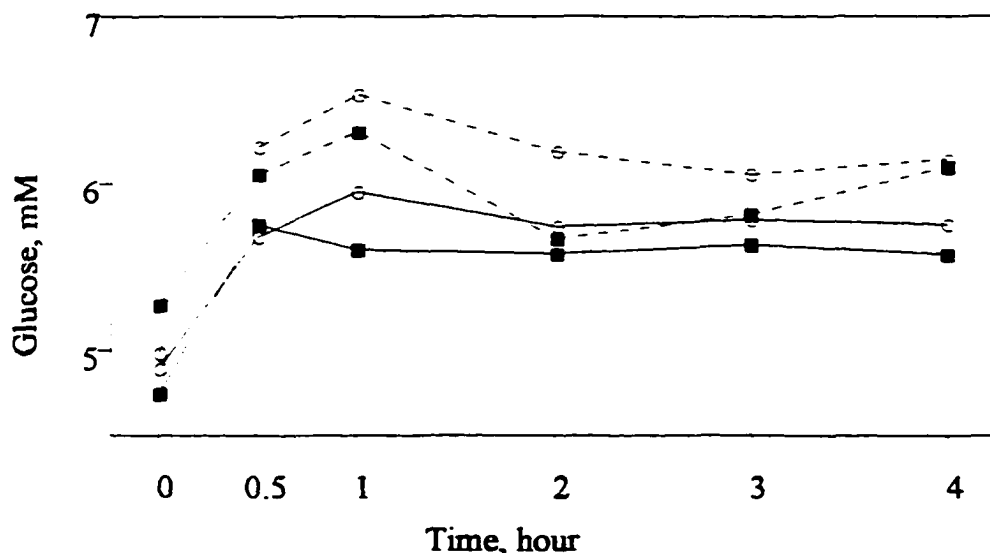


Figure 4.10. Changes in plasma glucose concentrations relative to a milk feeding at 5 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

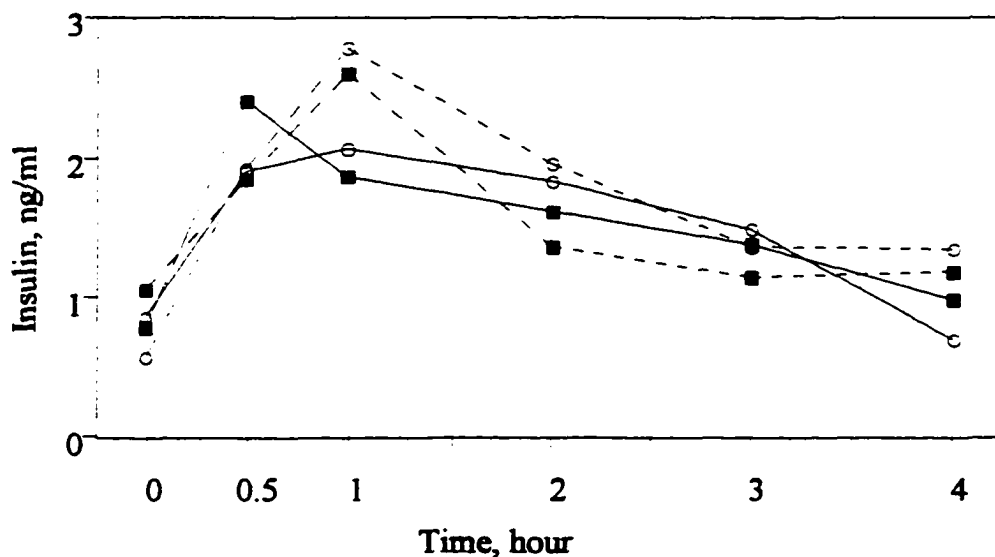


Figure 4.11. Changes in plasma insulin concentrations relative to a milk feeding at 5 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

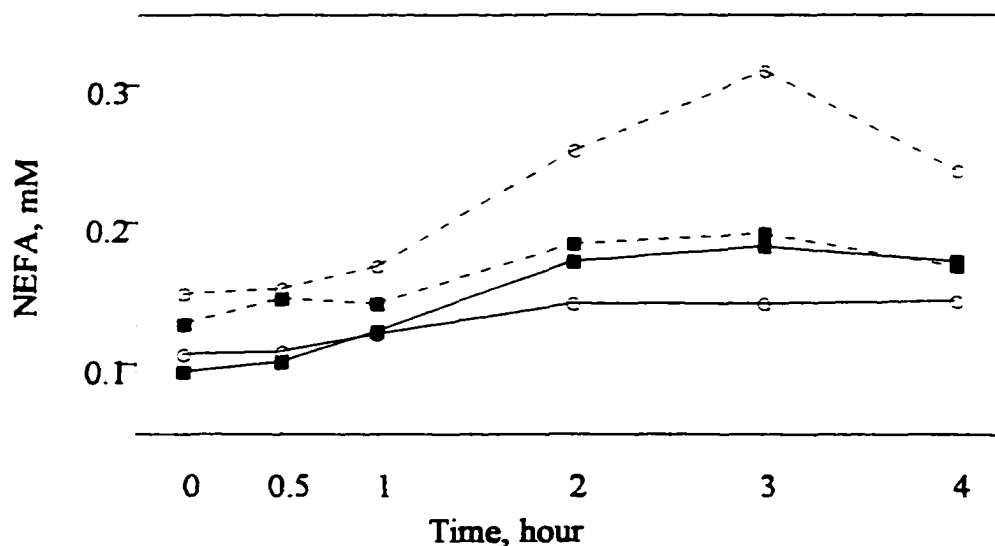


Figure 4.12. Changes in plasma nonesterified fatty acid (NEFA) concentrations relative to a milk feeding at 5 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

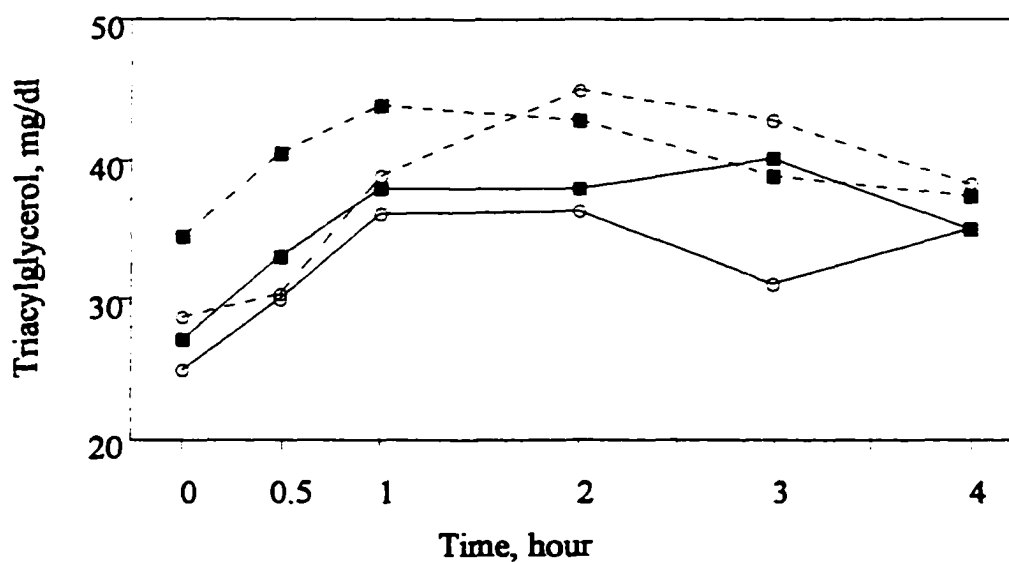


Figure 4.13. Change in plasma triacylglycerol concentrations relative to a milk feeding at 5 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

Table 4.5. Effect of chromium nicotinate (CrNIC) and fat level on plasma glucose, insulin, nonesterified fatty acids (NEFA) and triacylglycerol responses relative to consumption of milk replacer in 5 week old pre-weaned, conventionally-fed Holstein calves<sup>1</sup>.

Item	Treatment			P-value <sup>2</sup> for Treatment	Fat Level			P-value <sup>2</sup> for Fat Level
	Control	CrNIC	SEM		Basal	High	SEM	
n	17	17			16	18		
<b>Glucose</b>								
Basal concentration, mM <sup>3</sup>	5.0	5.0	.15	.79	4.8	5.2	.15	.13
Peak concentration, mM <sup>4</sup>	6.5	6.6	.18	.90	6.3	6.9	.18	.02
Response curve area, h-mM <sup>5</sup>	2.9	4.0	.53	.17	3.3	3.5	.53	.81
<b>Insulin</b>								
Basal concentration, ng/ml	1.1	.92	.20	.46	1.0	1.0	.20	.83
Peak concentration, ng/ml	3.1	3.5	.55	.63	2.9	3.7	.55	.29
Response curve area, h-ng/ml	2.6	5.0	1.20	.28	3.1	4.4	1.16	.97
<b>NEFA</b>								
Basal concentration, mM	.12	.15	.02	.36	.11	.16	.02	.06
Peak concentration, mM	.23	.27	.02	.14	.21	.29	.02	.008
Response curve area, h-mM	.20	.19	.05	.87	.16	.22	.05	.40
<b>Triacylglycerol</b>								
Basal concentration, mM	32.7	27.9	3.2	.29	27.7	33.0	3.1	.25
Peak concentration, mM	52.6	49.1	3.3	.46	48.1	53.7	3.3	.25
Response curve area, h-mM	32.3	40.1	8.4	.51	36.7	35.6	8.4	.93

(Table con'd)

<sup>1</sup>There were no (  $P > .10$ ) dietary treatment interactions, except for prefeeding plasma insulin (treatment x fat;  $P = .10$ ) and peak plasma NEFA concentrations (treatment x fat;  $P = .004$ ).

<sup>2</sup>Probability of a greater F-value.

<sup>3</sup>Basal concentrations = one sample immediately prior to offering milk.

<sup>4</sup>Highest point following consumption of milk.

<sup>5</sup>Response curve area = area-under the response curve relative to basal levels computed using trapezoidal geometry from the time period 0 to 4 h after offering milk.

the rise in plasma NEFA concentration was much more dramatic in the Cr-fed compared with the control-fed calves. Prefeeding plasma NEFA concentrations were greater ( $P = .06$ ; Table 4.5) for calves fed HF replacer compared with the calves fed the LF replacer. When calves were fed the BF milk replacer, peak postfeeding plasma NEFA concentrations were lower in calves receiving the Cr-supplemented replacer compared with calves receiving the control replacer; whereas, when calves were fed the HF milk replacer, peak postfeeding plasma NEFA concentrations were higher in calves receiving the Cr-supplemented replacer compared with the calves receiving the control replacer ( $P = .004$ ; Table 4.5). Area under the response curve for plasma NEFA was not affected ( $P > .10$ ; Table 4.5) by treatment. For triacylglycerol, there were no fat by time interactions ( $P = .96$ ; Figure 4.13) and none of the response variables were affected by the fat level of the milk replacer ( $P > .10$ ; Table 4.5).

Changes in Plasma Metabolites and Hormones During an Intravenous Glucose Tolerance Test. The changes over time and selected response variables (pre-injections concentrations, clearance rates, and area under the curve) for glucose and insulin during the IVGTT are presented in Figures 4.14 and 4.15 and Table 4.6, respectively. Pre-injection plasma glucose and insulin concentrations were similar for the dietary treatments ( $P > .10$ ; Table 4.6), and no independent dietary treatment by time interactions ( $P > .10$ ; Figures 4.14 and 4.15) were observed. The Cr by fat by time three-way interactions for glucose ( $P = .13$ ) and insulin ( $P = .19$ ) were also not significant. However, both metabolites responded in a similar manner. When the area under the glucose response curves were calculated from 0 to 60 and from 0 to 120 min post-injection, no dietary treatment differences were observed



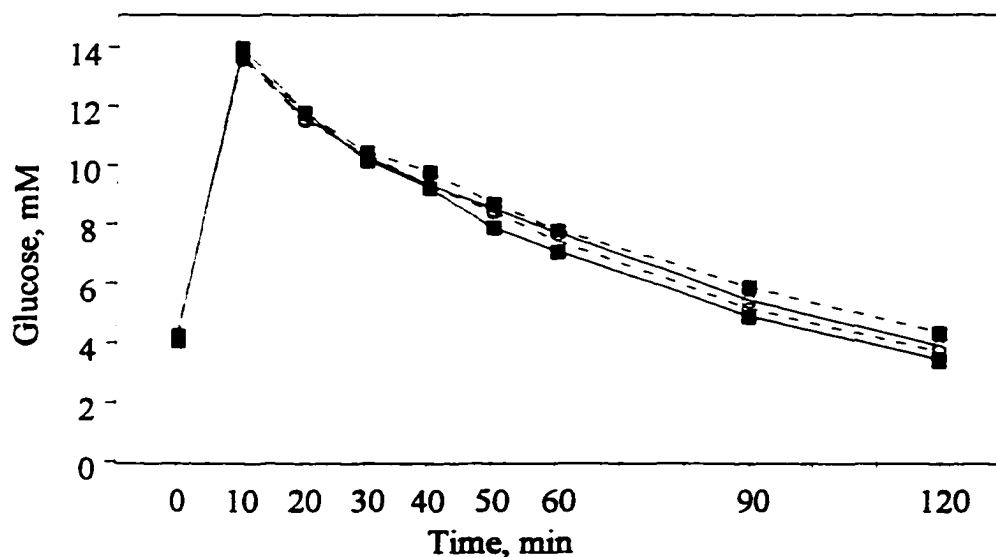


Figure 4.14. Changes in plasma glucose concentrations during an i.v. glucose tolerance test conducted at 5 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

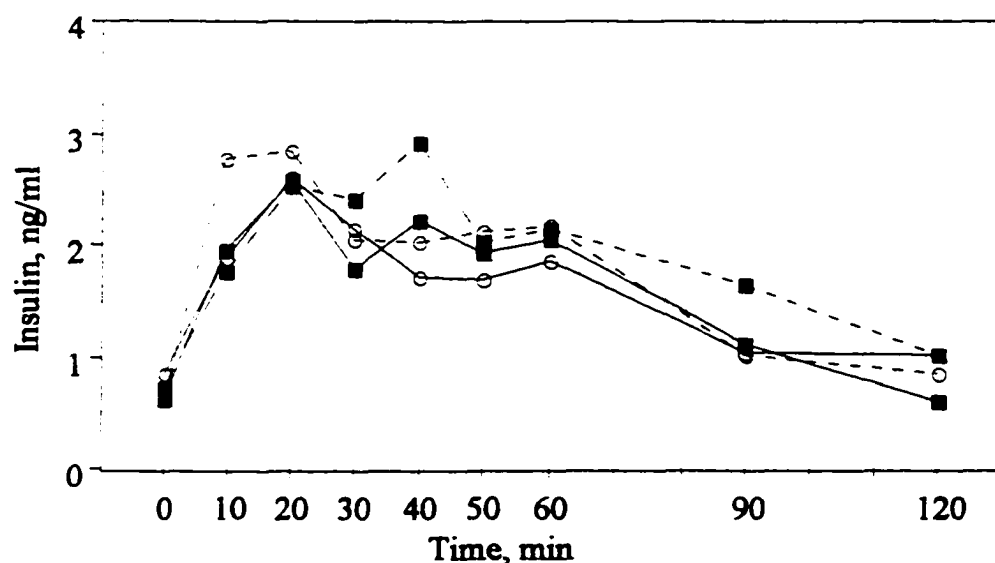


Figure 4.15. Changes in plasma insulin concentrations during an i.v. glucose tolerance test conducted at 5 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

**Table 4.6. Effect of chromium nicotinate (CrNIC) and fat level on kinetic response variables<sup>1</sup> during an i.v. glucose tolerance test in 5 week old pre-weaned, conventionally-fed Holstein calves<sup>2</sup>.**

Item	Treatment			P-value <sup>3</sup> for treatment	Fat Level			P-value <sup>3</sup> for treatment
	Control	CrNIC	SEM		Basal	High	SEM	
<b>n</b>	17	17			16	18		
<b>Glucose Kinetics</b>								
Basal concentrations, mM	4.2	4.2	.10	.73	4.1	4.2	.10	.37
Clearance rate, %/min <sup>4</sup>	1.10 (.89, 1.24) <sup>5</sup>	1.01 (.81, 1.24)		.57	1.15 (.93, 1.40)	.97 (.77, 1.18)		.24
Response curve area, min·mM Post-challenge interval								
0 to 60 min	345	344	10.4	.93	345	344	10.5	.94
0 to 120 min	431	426	26.2	.90	424	433	26.0	.80
<b>Insulin Kinetics</b>								
Basal concentrations, ng/ml	.79	.81	.09	.84	.83	.76	.08	.57
Clearance rate, %/min	.95	1.20	.55	.74	1.20	.95	.55	.75
Response curve area, min·ng/ml Post-challenge interval								
0 to 60 min <sup>6</sup>	91 (68, 124)	78 (58, 106)		.45	74 (54, 101)	97 (72, 130)		.20
0 to 120 min <sup>6</sup>	140 (108, 182)	112 (87, 146)		.22	106 (81, 138)	149 (116, 192)		.07
Insulin Sensitivity Index <sup>7</sup>	2.45 (1.59, 3.50)	3.76 (2.63, 5.09)		.09	4.08 (2.87, 5.50)	2.21 (1.14, 3.18)		.02

(Table con'd)

<sup>1</sup>Kinetic criteria: basal concentrations immediately prior to challenges; clearance rate computed from 20 to 50 min after glucose challenge.

<sup>2</sup>There were no ( $P > .10$ ) dietary treatment interactions.

<sup>3</sup>Probability of a greater F-value.

<sup>4</sup>For statistical analysis, glucose clearance rates were transformed by raising the original values to the .5 power.

<sup>5</sup>For glucose clearance rate, insulin response curve area, and insulin sensitivity index, all means are based on analysis of transformed data; consequently, the lower and upper 95% confidence limits for each mean are given in parentheses.

<sup>6</sup>For statistical analysis, insulin response curve areas were transformed by taking the natural log of the original values.

<sup>7</sup>Based on a computer model (Bergman, 1997): larger number indicates = more insulin sensitive: Insulin sensitivity indexes were transformed by raising the original values to the .5 power.

( $P > .10$ ; Table 4.6). During the initial h post-injection (0 to 60 min), insulin AUC was also not affected ( $P > .10$ ) by dietary treatments; however, over the 2-h sampling period (0 to 120 min), area under the insulin response curve was greater ( $P = .07$ ) in calves fed the HF replacer compared with those fed the BF replacer. Similar responses have been observed in milk-fed calves (Kegley et al., 1997a) and in ruminating lambs (Fornea et al., 1994; Kitchalong et al., 1995; Forbes et al., 1998) and calves (Bunting et al., 1994).

Glucose and (or) insulin clearance rates following an IVGTT or an IVICT have been the primary diagnostic tool used to evaluate whether supplemental Cr affected glucose tolerance/insulin sensitivity. In the current experiment, glucose and insulin clearance rates were not affected ( $P > .10$ ; Table 4.6) by the dietary treatments. Kegley et al. (1997a) also reported that supplemental Cr had no effect on glucose clearance rate following an IVGTT conducted in 9 to 10-wk old intensively-fed calves. Fornea et al. (1994), Kitchalong et al. (1995), and Forbes et al. (1998) have also reported no effect of supplemental Cr-picolinate on glucose clearance rates in lambs administered an IVGTT. Bunting et al. (1994) reported supplemental Cr (as Cr-picolinate) increased glucose clearance rates by 40% in steers and by 27% in heifer calves during an IVGTT. It is noteworthy that the clearance rates were faster for the older, milk-fed calves (fed a 20% fat milk replacer was fed) used by Kegley et al. (1997a) compared to the younger, conventionally-fed calves (fed on average a 19% fat milk replacer) used in the current experiment (average of all treatments = 1.59 vs 1.06 %/min, respectively).

As mentioned in the material and methods, the plasma glucose and insulin concentrations obtained from the IVGTT were also evaluated by a computer modeling

procedure that allowed us to obtain an index of insulin-dependent glucose clearance (insulin sensitivity index). A higher index value indicates that the subject is more insulin sensitive. The insulin sensitivity index values obtained were similar to those that have been reported for non-insulin dependent diabetic human subjects (Watanabe et al., 1995) and for humans fed a low fat compared to a high fat diet (Lovejoy, unpublished). More classical methods, such as the pancreatic suppression test and glucose clamps, have been compared to the minimal model approach. All methods used have shown that insulin sensitivity decreases as the level of dietary fat in the diet increases (Sidery et al., 1990; Harris and Kor, 1992). For the current experiment, both Cr level and dietary fat level had independent effects on the insulin sensitivity index. Calves fed the Cr-supplemented replacer had higher ( $P = .09$ ) index values than calves fed the control replacer (3.76 vs 2.45, respectively) and calves fed the BF replacer had higher ( $P = .02$ ) index values than calves fed the HF replacer (4.08 vs. 2.21, respectively). The fat effect supports our original hypothesis that feeding calves a higher level of dietary fat would have reduced insulin sensitivity. This Cr effect is similar to that of Kegley et al. (1997a) where they suggested that Cr supplementation may prolong insulin sensitivity prior to the time calves are weaned.

#### Changes in Plasma Metabolites and Hormones During an Epinephrine Challenge.

Changes plasma concentrations over time and selected response variables (pre-injection concentrations and area under the curve) for glucose, NEFA, and triacylglycerol during the epinephrine challenge are represented in Figures 4.16 through 4.18, and Table 4.7. Pre-injection plasma glucose, NEFA, and triacylglycerol concentrations did not differ between treatments ( $P > .10$ ; Table 4.7); however, there was a tendency for plasma triacylglycerol

concentrations to be greater ( $P = .11$ ; Table 4.7) in calves fed the HF-replacer compared with calves fed the LF-replacer. Plasma glucose, NEFA, and triacylglycerol concentrations all peaked within 10 min after the epinephrine was given ( $P = 0001$ ). Plasma glucose and NEFA concentrations returned to pre-injection levels by around 60 min and remained stable over the ensuing h. For all dietary treatments, a similar pattern was observed for plasma triacylglycerol concentrations throughout the first 40 min post-injection; however, after plasma triacylglycerol concentrations reached their lowest post-injection point, concentrations began to increase in the control-fed calves but remained relative stable in the Cr-fed calves. The triacylglycerol area under the curve from 0 to 60 min did not differ ( $P = .22$ ) between Cr-fed and control-fed calves; but, over the entire 120 min sampling period, there was a slight tendency ( $P = .15$ ) for area under the curve to be lower in Cr-fed compared with the control-fed calves (234 vs 506, respectively). Because epinephrine is a potent catecholamine (Brockman and Laarveld, 1986) and catecholamines have been shown to stimulate glycogenolysis, gluconeogenesis, and lipolysis in ruminants and nonruminants (Blum et al., 1982; Mersmann, 1989; Chapa et al., 1996), the rise in glucose, NEFA, and triacylglycerol was expected. Based on the current findings, it is possible that Cr helps to stabilize plasma lipid concentrations.

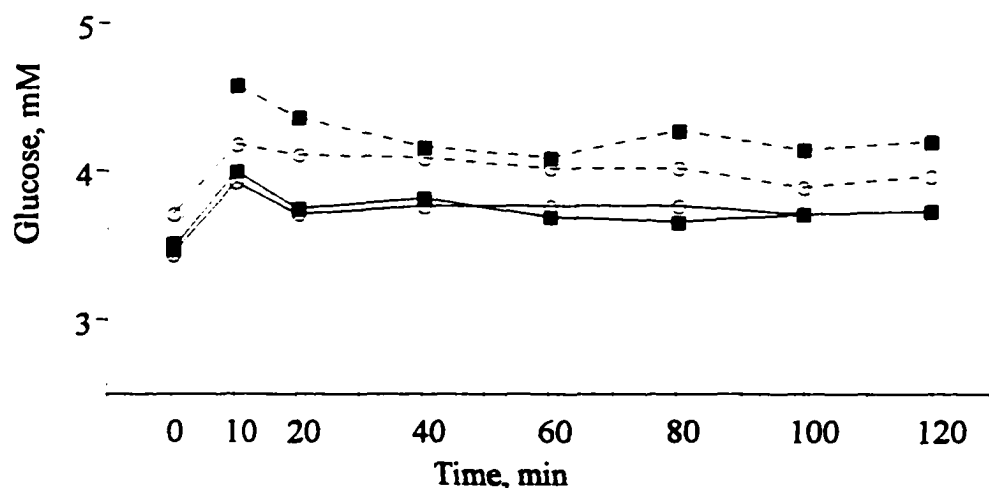


Figure 4.16. Changes in plasma glucose concentrations relative to an epinephrine infusion at 5 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

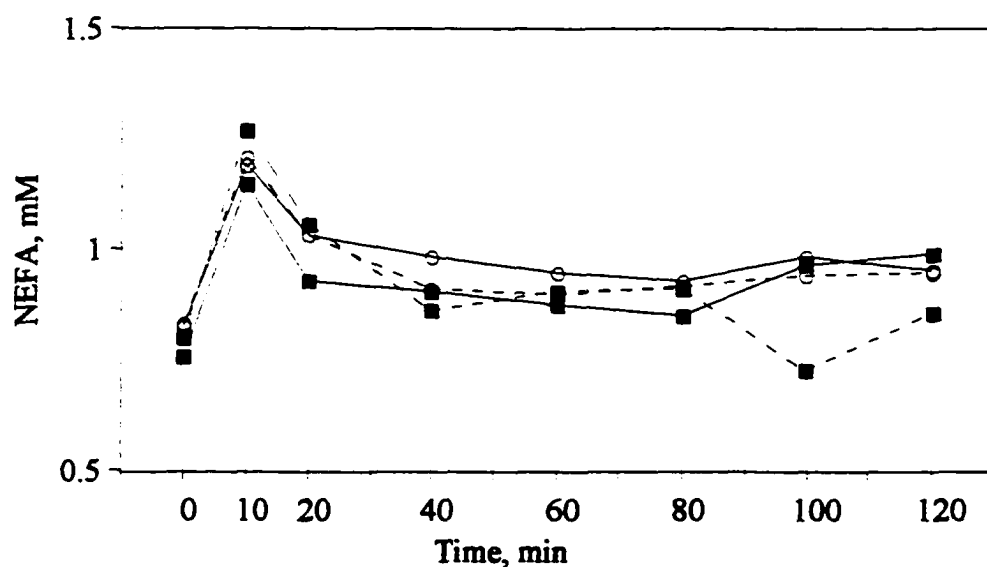


Figure 4.17. Changes in plasma nonesterified fat acid (NEFA) concentrations relative to an epinephrine infusion at 5 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.

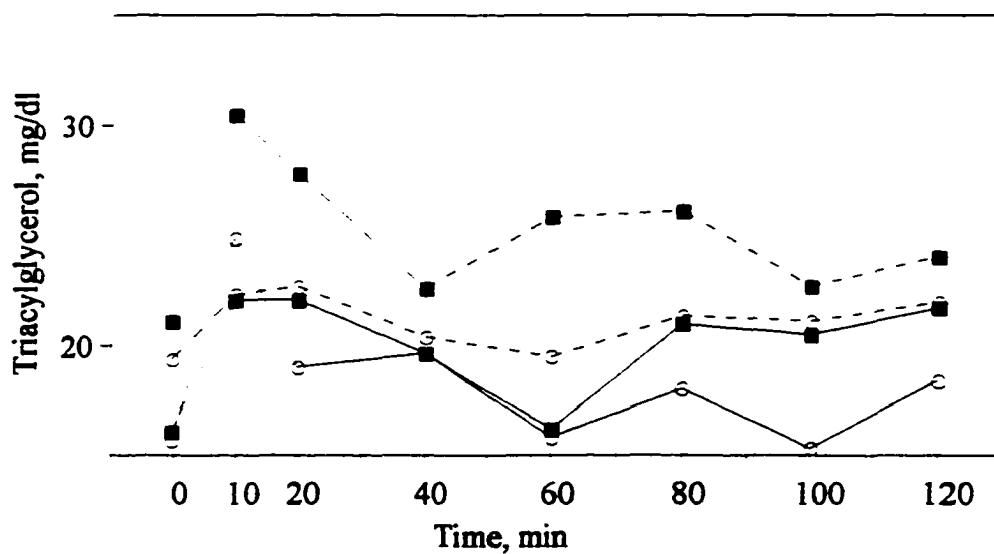


Figure 4.18. Changes in plasma triacylglycerol concentrations relative to an epinephrine infusion at 5 weeks of age. Calves were fed milk replacer that contained either 15% (basal) or 22% (high) fat without (control) or with supplemental chromium nicotinate (CrNIC). Solid line = basal fat; dash line = high fat; square = control; circle = CrNIC.



**Table 4.7. Effect of chromium nicotinate (CrNIC) and fat level on kinetic response variables<sup>1</sup> during an i.v. epinephrine infusion in 5 week old pre-weaned, conventionally-fed Holstein calves<sup>2</sup>.**

Item	Treatment			P-value <sup>3</sup> for treatment	Fat Level			P-value <sup>3</sup> for Fat Level
	Control	CrNIC	SEM		Basal	High	SEM	
<b>n</b>	<b>17</b>	<b>17</b>			<b>16</b>	<b>18</b>		
<b>Glucose Kinetics</b>								
Basal concentration, mM	3.49	3.58	.13	.66	3.46	3.62	.13	.40
Response Curve area, min·mM								
Post-challenge interval								
0 to 60 min <sup>4</sup>	25.3 (16.5, 36.2) <sup>5</sup>	16.6 (9.5, 25.4)		.16	15.3 (8.4, 24.2)	26.9 (18.0, 37.7)		.07
0 to 120 min <sup>4</sup>	42.8 (22.1, 68.7)	30.7 (12.7, 53.7)		.43	27.7 (9.9, 50.7)	46.3 (25.4, 72.1)		.23
<b>NEFA Kinetics</b>								
Basal concentration, mM	.78	.83	.06	.61	.79	.82	.06	.81
Response Curve area, mM·min								
Post-challenge interval								
0 to 60 min <sup>4</sup>	7.7 (3.0, 13.6)	9.3 (4.2, 15.4)		.69	10.0 (4.7, 16.5)	7.1 (2.6, 12.6)		.44
0 to 120 min	16.6	17.0	5.5	.96	19.5	14.0	5.5	.48
<b>Triacylglycerol Kinetics</b>								
Basal concentration, mg/dl	19.0	18.7	2.0	.91	16.5	21.2	2.0	.11
Response Curve area, mM·min								
Post-challenge interval								
0 to 60 min	256	163	53.2	.22	217	303	53.3	.86
0 to 120 min	506	234	129.9	.15	390	349	128.8	.82

(Table con'd)

<sup>1</sup>Kinetic criteria: basal concentrations are average of 2 samples taken 10 min and immediately prior to challenge.

<sup>2</sup>There were no ( $P > .10$ ) dietary treatment interactions.

<sup>3</sup>Probability of a greater F-value.

<sup>4</sup>For statistical analysis, glucose (0 to 60 and 0 to 120 min) and insulin (0 to 60 min) response curve areas were transformed by raising the original values to the .4 power.

<sup>5</sup>For glucose (0 to 60 and 0 to 120 min) and insulin (0 to 60 min) response curve areas, all means are based on analysis of transformed data; consequently, the lower and upper 95% confidence limits for each mean are given in parentheses.

## CHAPTER V

### OVERALL SUMMARY AND CONCLUSIONS

Two experiments were conducted to determine the effects of supplemental chromium (Cr; 1 ppm), as chromium picolinate (CrP; exp. 1) or chromium nicotinate (CrNIC; exp. 2), on performance, glucose metabolism, and lipid metabolism of conventionally-fed Holstein calves.

Supplemental CrP (Exp. 1; between birth and 2 to 3 wk post-weaning) was observed to increase ADG in bull calves but to decrease ADG in heifer calves. Additionally, modest increases in ADG (Exp. 2) were observed while calves were fed milk alone. From these experiments, it was concluded that performance benefits alone will not justify usage of supplemental Cr in conventionally-reared dairy calves.

In cattle, research efforts have identified two situations in which Cr supplementation might have a commercial application: newly arrived feedlot cattle and first-lactation dairy cattle during the transition period. In swine, research has determined, that under some situations, Cr supplementation might favorably alter metabolism with resultant improvements in performance. Overall, it appears that supplemental Cr tends to improve performance in situations in which demands (nutritional, metabolic, environmental, etc.) placed on the animal are high. It is possible that the effects of Cr on performance were minimal in our experiments, because the demands imposed on the calves were reduced through optimum calf management and husbandry practices. For instance, in the current experiments, care was taken to ensure that adequate amounts of high quality colostrum was provided to all calves in a timely manner. Additionally, only calves readily drinking from

buckets and not displaying any apparent health problems were included in the current experiment. These are probably the primary reasons that the incidence and severity of calf scours were low and that the rates of gain tended to exceed those reported for calves of similar ages, and when similar feeding regimes were employed. Heinrichs et al. (1994) reported that 25.6% and 75.5% of calves on US farms receive less than 1.9 L and less than 3.8 L of colostrum within the first 24 h of life, respectively. Even when an acceptable quality of colostrum ( $> 50$  mg of immunoglobulins/mL of colostrum; Scott and Fella, 1983) is used it has been determined that calves receiving less than 1.9 L of this colostrum will not receive an adequate level of immunoglobulins (Scott et al., 1981). Calves receiving between 1.9 and 3.8 L also may not receive adequate immunoglobulins (Heinrichs et al., 1994). Whether Cr supplementation would have greater effects on performance (gain, health) in dairy calves, raised under less than ideal calf management practices, warrants investigation.

From Exp. 1 and 2, it was concluded that supplemental Cr had limited and (or) inconsistent effects on the metabolites and hormones measured before (prefeeding) and after milk feedings. Supplemental CrP did not affect prefeeding plasma glucose, insulin, cortisol, or IGF-1 concentrations and did not affect the postfeeding rise in plasma glucose or insulin concentrations. Supplemental CrNIC did not affect prefeeding plasma glucose, insulin, triacylglycerol, or cholesterol concentrations, although there was a tendency for CrNIC to lower prefeeding plasma insulin concentrations. Regarding NEFA, supplemental CrP lowered pre- and postfeeding plasma NEFA concentrations in Exp. 1, whereas in Exp. 2, prefeeding plasma NEFA concentrations were greater when CrNIC was supplemented. This

latter effect was largely because plasma NEFA concentrations in the calves fed the CrNIC, high fat-replacer did not decrease with age to the extent observed for the other treatment groups. From Exp. 2, basal cholesterol and triacylglycerol (under some situations) also tended to show that fat level of the diet affected how these metabolites responded to supplemental Cr. In response to milk feedings (Exp. 2 only), CrNIC and fat level had only small effects on the blood metabolites measured; however, the findings did suggest that fat level of the diet needs to be considered when evaluating blood metabolite responses to supplemental Cr.

From Exp. 1, it was concluded that supplemental CrP did not affect gluconeogenesis. From Exp. 1 and 2, it was concluded that supplemental Cr did not affect glucose and insulin changes over time or glucose clearance rates in response to an IVGTT. However, interpretation of the IVGTT data by the minimal model program (Exp. 2 only), showed that calves fed CrNIC were more insulin sensitive than control-fed calves and that BF-fed calves were less insulin sensitive than HF-fed calves. Further research needs to be conducted to determine if use of the minimal model approach would provide a better evaluation of changes in insulin sensitivity in the neonatal calf.

Many unknown factors remain in Cr research. The need for Cr supplementation of practical livestock diets appears to depend on the Cr status of the animal, the amount of bioavailable Cr in feedstuffs, and the exposure of the animals to situations under which greater demands are placed on the animal. To date, few studies, especially in ruminants, have been conducted to compare different Cr sources and (or) levels supplemented.

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## VITA

Christopher L. DePew was born on December 20, 1967, in Goshen, Indiana, to Larry and Peg DePew. He graduated from West Noble High School in 1986 and then spent one year at Anderson College, Anderson, Indiana. He transferred to Purdue University and received his bachelor of science degree in Animal Science in December, 1990. He then began work toward a master of science degree at Louisiana State University in January of 1991. Upon completion of his master's degree in Animal Science in May 1993, he began work toward a doctor of philosophy degree in Dairy Science at Louisiana State University. Following completion of this degree, he plans to find an industry position in research and development, where he can be involved in the development of products that will be of benefit to animal producers.

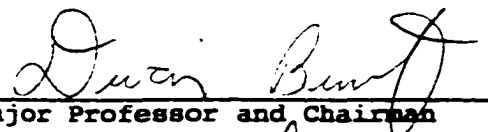
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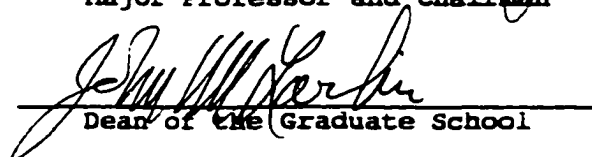
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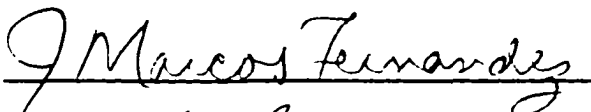
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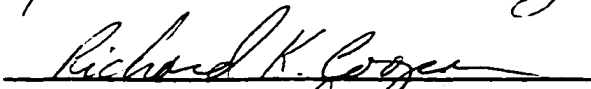
  
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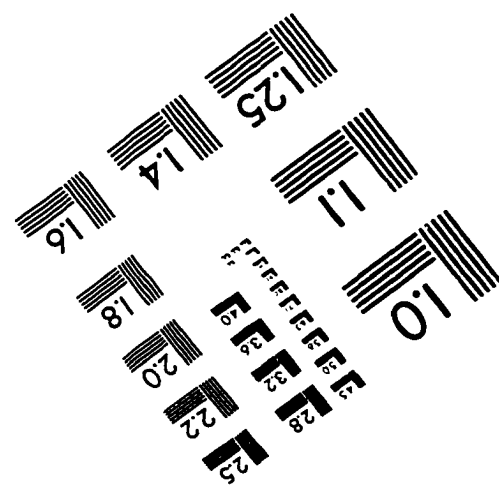
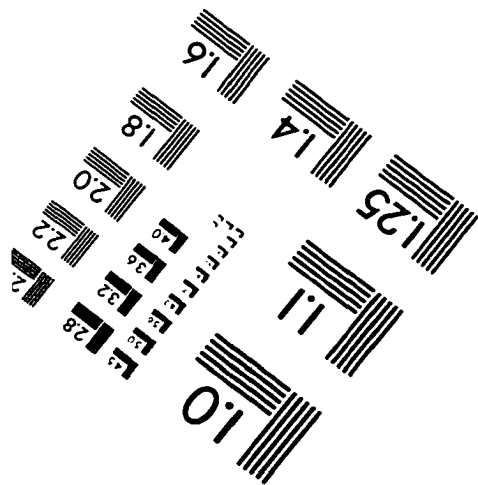
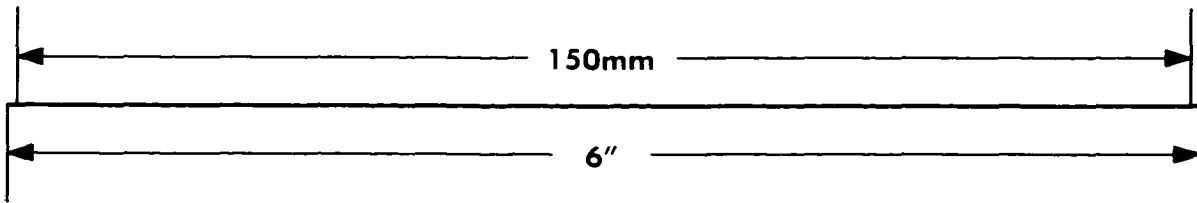
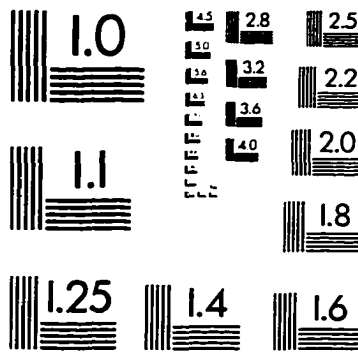
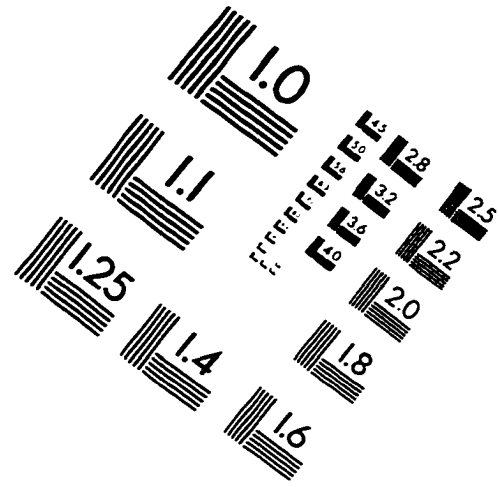
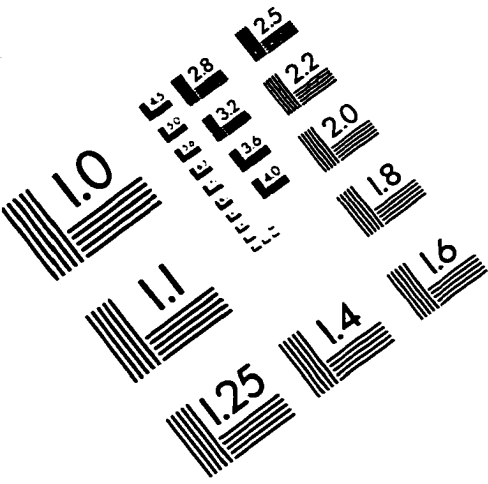




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